# EVALUATING ENDOGENOUS CARBON MONOXIDE (CO) PRODUCTION AS AN INDICATOR FOR PULMONARY FUNCTION TESTING (PFT)

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## ABSTRACT

Chronic lung diseases, such as chronic obstructive pulmonary disease (COPD) and asthma, are associated with considerable morbidity and mortality in the United States. COPD is currently the 4th leading cause of death in the United States and a major cause of morbidity. More than 26 million Americans have asthma, which accounts for 14.2 million doctor's office visits each year, according to the Centers for Disease Control and Prevention (CDC). These lung diseases involve chronic inflammation and oxidative stress. However, the diseases are not diagnosed and treated efficiently in routine clinical practice because of the difficulties in monitoring inflammation. Consequently, it is often too late to alter respiratory dysfunction when patients' referral for pulmonary function testing is delayed.

The purpose of this descriptive-correlational study was to explore the possible associations between pulmonary function test (PFT) measurements and exhaled carbon monoxide (eCO) levels as an indicator of generalized inflammation. Data analysis was conducted to determine a potential level of eCO to use as an indicator for conducting PFT. The contributions of demographic factors, patient history of smoking habits and drug use, and the medical diagnosis to the prediction of principal variables were also investigated.

A sample of 343 subjects, who were referred by their physicians for a routine care PFT, were recruited from the Queen's Medical Center Pulmonary Lab in Honolulu, Hawaii. A study-specific information sheet was provided to the subjects as part of the informed consent process, and the subjects' data were collected through a Demographic Data Questionnaire. Exhaled carbon monoxide (eCO) levels were measured with a portable carbon monoxide analyzer (MicroCO Meter), and the maximum values from three successive measurements were used in all calculations.

Carbon monoxide was detectable and measured reproducibly in the exhaled air of all subjects. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC, and forced expiratory flow 25-75% ($FEF_{25-75\%}$) decreased with elevated concentrations of eCO. In females, decreased lung volumes (total lung capacity, functional residual capacity, and residual volume) were associated with increased eCO levels. In males, increased lung volumes were associated with increased

eCO levels. Diffusion capacity of lungs for carbon monoxide (DLCO) and eCO levels also showed the opposite correlation between females and males. DLCO of female subjects markedly decreased with increased levels of eCO while DLCO of male subjects mildly increased.

Appropriate cut-off points of eCO levels also were examined to determine the most efficient use of eCO as an indicator for PFT. The present study found that a cut-off point for eCO of 6 ppm provided the best relationship between sensitivity and specificity in predicting the need for PFT.

In conclusion, eCO measurement, which is noninvasive, quick, inexpensive, and easily administered by primary care physicians, could serve as a useful biomarker for monitoring patients with pulmonary diseases. Therefore, eCO measurement may be clinically useful as a diagnostic tool to identify inflammation and to serve as an indicator of the need to conduct PFT.

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| | |
|---|---|
| BMI | Body mass index |
| CDC | Centers for Disease Control and Prevention |
| cGMP | Cyclic Guanosine monophosphate |
| COHb | Carboxyhemoglobin |
| COPD | Chronic obstructive pulmonary disease |
| DLCO | Diffusion capacity for carbon monoxide |
| EC | Enzyme commission of the international union of biochemistry |
| eCO | Exhaled carbon monoxide |
| Fe2+ | Ferrous iron |
| FEF25-75% | Forced Expiratory Flow25-75% |
| FEV1 | Forced Expiratory Volume |
| FRC | Functional Residual Capacity |
| FVC | Forced Vital Capacity |
| GOLD | Global Initiative for Chronic Obstructive Lung Disease |
| Hb | hemoglobin |
| HO | heme oxygenase |
| IFN | Interferon |
| IL | Interleukin |
| ILD | Interstitial lung disease |
| kDa | Kilodaltons |
| N | Number |
| NHANES | National Health and Nutrition Examination Survey |
| PEF | Peak Expiratory Flow |
| PFT | Pulmonary Function Test |
| PI | Principal investigator |
| PPM | Parts per million |
| ROS | Reactive oxygen species |
| RV | Residual Volume |
| SD | Standard Deviation |
| SEM | standard error of the mean |
| TLC | Total Lung Capacity |
| TNF | Tumor necrosis factor |
| VC | Vital Capacity |
| WHO | World Health Organizations |

# CHAPTER I: LITERATURE REVIEW

## Chronic Respiratory Diseases and Inflammation

Chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD) and interstitial lung diseases are associated with considerable morbidity and mortality in both developed and developing nations worldwide (Chang, 2013). For example, asthma, which is one of the most common chronic lung diseases and characterized by chronic inflammation and recurrent exacerbations, affects 26 million people in the United States (CDC, 2018) and an estimated 300 million people worldwide (Masoli et al., 2004). The WHO Global Burden of Disease estimated that there are more than 17 million Americans have COPD, the fourth leading cause of death in the United States and a major cause of morbidity, and COPD will be the third leading cause by 2020. COPD is also associated with significant economic burden, and the estimated direct costs of COPD are $32 billion in the United States (GOLD, 2014).

Inflammation caused by oxidative stress has an important role in the pathogenesis and progression of chronic lung diseases. For example, asthma is a condition of inflammation predominantly in the airways (Bousquet et al., 2000), and COPD is affected by chronic inflammation in the regions of airways and lung parenchyma that includes bronchioles and alveoli (Rennard, 1999).

Biological systems are continuously exposed to oxidants that can be either generated endogenously by metabolic reactions, such as mitochondrial electron transport during respiration, or exogenously by inhaled chemical particles, such as air pollutants or cigarette smoke (MacNee, 2001). Increased oxidative stress results in lung inflammation in the airways (Dworski, 2000). For example, when asthmatic patients are exposed to oxidants, the inflammatory and immune cells in the airways, such as macrophages, neutrophils, and

eosinophils, release increased amounts of reactive oxygen species (ROS), such as superoxide anion ($\cdot O_2^-$) and the hydroxyl radical ($\cdot OH$). ROS are unstable molecules with unpaired electrons and are capable of initiating oxidation and triggering inflammation which produces asthmatic symptoms such as bronchospasm or increased mucus production (Calhoun et al., 1992; Kanazawa et al., 1991; Sedgwick et al., 1990).

Primary care physicians are frequently the entry point into the health care delivery system and often manage patients with chronic respiratory symptoms due to lung inflammation, and they need to establish a diagnosis to proceed to offer treatment. The diagnosis of chronic respiratory diseases typically consists of a comprehensive assessment of patients' symptoms, pulmonary function testing, and radiological imaging (Vestbo, et al, 2013).

**Pulmonary Function Test**

The most important function of the lungs is the gas exchange, adding oxygen ($O_2$) and removing excess carbon dioxide ($CO_2$) when mixed venous blood passes through the pulmonary circulation. The ability of the lungs to perform gas exchange depends on three physiologic functions: 1) the thorax must expand by the diaphragm and thoracic muscles to produce a subatmospheric pressure so that air can flow into the lungs; 2), the airways must be unobstructed; 3) the alveolar-capillary membrane should be undamaged so that $O_2$ and $CO_2$ can diffuse through.

Pulmonary function testing (PFT) can provide valuable information about these important individual processes for gas exchange. Various measurements are available to aid in the diagnosis and assessment of pulmonary diseases, to determine the need for therapy, and to evaluate the effectiveness of respiratory care (Kacmarek, Stoller, & Heuer, 2017). Pulmonary function test is typically indicated in patients with respiratory dysfunction symptoms such as

cough, wheezing, or shortness of breath, history of cigarette smoking, occupational exposures, or those who are planning to undergo abdominal and thoracic procedures. The goal of pulmonary function testing is to provide information about the severity of symptoms and management effectiveness (Downs, 2011). PFT assists physicians to diagnose respiratory conditions, measure the disease progression, and initiate proper treatment.

PFT consists of three examinations: spirometry, lung volumes, and diffusing capacity of lungs for carbon monoxide (DLCO). These tests measure physical and functional properties, such as lung mechanics, airflow, and gas exchange (Khatri, 1994). Spirometry is the most frequently performed pulmonary function test. It measures the movement of air, speed and volume, into and out of the lungs during various breathing maneuvers. It can be used to confirm airway obstruction and to demonstrate reversibility of obstruction with bronchodilator medication. Spirometry is commonly used as a preferred diagnostic testing method not only to measure expiratory volume and flow but also to monitor the effectiveness of chronic therapy. Spirometry is recommended for all patients to confirm the diagnosis of asthma before initiation of possibly lifelong therapy (Petty, 2001; Bellamy, Booker, Connellan, & Halpin, 2005). Spirometry testing should be readily available and routinely used in medical offices and hospitals where patients have heart and lung diseases (Crapo, 1994). Lung volume testing is useful to determine the presence of a restrictive ventilatory defect and helps determine the degree of hyperinflation and air-trapping. DLCO has many indications, including differential diagnosis in restrictive and obstructive diseases and measures the transfer of a diffusible gas, which is carbon monoxide (CO), across the alveolar-capillary membrane. Interpretation of diffusing capacity tests should include appropriate adjustments for factors such as hemoglobin (Hb) and carboxyhemoglobin (COHb) (Ruppel and Enright, 2012)

PFT is an important tool in the diagnosis and management of most respiratory conditions, particularly with regard to diseases such as asthma, COPD, and interstitial lung disease. During the last decade, there has been a great deal of interest in the detection of COPD with spirometry as the primary tool in primary care practice settings. However, in the United States, only about 25% of new cases of COPD have had spirometry (Ruppel and Enright, 2012). The primary indication for the majority of PFT performed in these adult laboratories is to document lung function in a patient with previously diagnosed respiratory disease. It is important to characterize the indications for the performance of PFT. However, despite the clinical importance of PFT, there is little information in the literature investigating indications for performance of PFT in practice (Pretto et al, 2013), and this makes management of chronic lung diseases difficult since physicians usually rely on indirect measurements of lung inflammation such as symptoms and PFT. Therefore, it is often too late to alter the dysfunction when patients complain of the symptoms such as shortness of breath and are sent to a pulmonary function testing lab because of the advanced stage of the disease.

**Inflammation and Elevated Endogenous Carbon Monoxide**

Recent studies have shown that the levels of exhaled carbon monoxide (eCO) are elevated when inflammation occurs in the lungs. Therefore, eCO may contain valuable molecular clues to lung cell function and can be used as a biological marker for inflammation (Zayasu et al., 1997; Horvath et al., 1998; Antuni et al., 1999; Kharitov et al., 2001; Montuschi et al., 2001; Zhang et al., 2010). Carbon monoxide is an odorless gas and is detectable in the exhaled air of a normal person. All organisms having heme produce CO endogenously, and the majority of it is produced as a by-product in a reaction of oxidative heme degradation catalyzed by microsomal heme oxygenase (HO; EC 1.14.99.3) (Tenhunen et al., 1969). In healthy

subjects, investigators have measured levels of exhaled CO from 1 to 8 ppm (Yamaya et al., 2001). There are two isoforms of heme oxygenase in humans: HO-1 and HO-2. HO-1 is an inducible form by inflammatory mediators and also known as heat shock protein 32-kDa (Keyse & Tyrrell, 1989; Choi and Alam, 1996). HO-2, a 33-kDa isoform, is constantly expressed in most tissues, especially in testes and brain (Maines, 1986; Paredi et al., 2002).

Studies demonstrate that heme oxygenase-1 (HO-1) activity is important in normal cell functions and in adaptation to stressful situations arising from widely different stimuli (Maines, 1986; Willis et al., 1996; Horvath et al., 2001). HO-1 is expressed mainly in epithelial and endothelial cells of the respiratory system (Paredi et al., 1999b), and it can be activated by inflammatory mediators, oxidants, or by pro-inflammatory cytokines: IL-1β, IL-6, IFN-γ, TNF-α; bacterial toxins; airway viral infection; nitric oxide; heme; hemin; ozone, hyperoxia, hypoxia; reactive oxygen species (superoxide, peroxynitrite, hydrogen peroxide, hydroxyl radical); and reactive nitrogen species (Horvath et al., 2001; Nath et al., 2001; Kharitonov & Barns, 2001).

Oxidative stress in cells increases heme degradation by increased activity of heme oxygenase-1 (HO-1), producing carbon monoxide (CO), ferrous ions, and biliverdin (Fig. 1). These by-products were viewed only as waste products, and it took a rather long time to discover their biological significance. It has been demonstrated that carbon monoxide produced during the heme degradation counteracts pro-inflammatory cytokine cascades by inhibiting the production of cytokines critical for T-cell responses. Indeed, Inoeue et al (2001) successfully demonstrated that increased expression of HO-1 by mouse macrophages or treatment of these cells with CO inhibits the production of pro-inflammatory cytokines. Therefore, the amount of exhaled CO (eCO) might reflect the level of HO-1 induction, which in turn reflects the severity of inflammation.

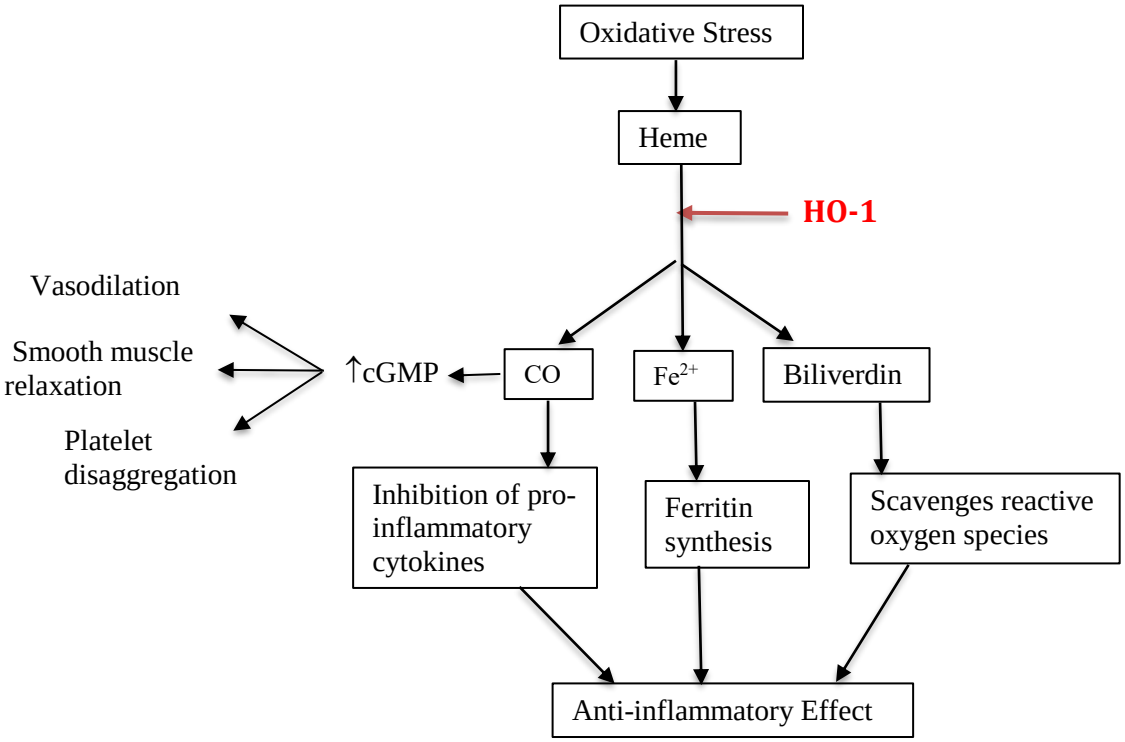



Figure 1. Pathway of heme metabolism catalyzed by heme oxygenase (Otterbein et al., 1999).

Carbon monoxide (CO) can also activate soluble guanylate cyclase, a critical enzyme involved in cell signaling as an intracellular messenger molecule. CO produced by HO-1 triggers increased expression of 3',5' guanosine monophosphate (cGMP) which results in vasodilatation, relaxation of smooth muscle, and thrombocyte disaggregation (Otterbein et al., 1999). Vasodilation induced by CO can be essential for adequate tissue perfusion and oxygenation under hypoxic conditions.

Biliverdin, produced during the heme degradation by HO-1, is converted quickly to bilirubin by biliverdin reductase. Recently, bilirubin and biliverdin have been shown to be highly efficient scavengers of reactive oxygen species, thus playing a role as effective antioxidants (Stocker et.al. 1987; Baranamo et al., 2002; Ohrui et al., 2003).

Released ferrous ions in the process of heme degradation induce ferritin synthesis. The increased production of ferritin provides cytoprotection since free iron is capable of extensive cellular damage by promoting the generation of reactive oxygen species through the Fenton reaction. Ferritin allows safe ferrous ion sequestration (Eisenstein et al., 1991). This evidence supports the notion that HO-1 is an essential component for the lung to protect against oxidative stress and subsequent oxidative damage.

Exhaled CO (eCO) is likely from a systemic elimination process through diffusion of CO from the pulmonary circulation through the alveoli. It has been proposed to use eCO as a practical tool for health professionals to assess and monitor smoking status (Ryter and Choi, 2013). Sato et al. (2003) showed that the eCO level was lower in ex-smokers with COPD than in current smokers with COPD. Studies have also investigated eCO to quantify inflammation in asthma, bronchiectasis, and cystic fibrosis patients who have higher CO levels than healthy subjects (Zayasu et al., 1997; Horvath et al., 1998a; Antuni et al., 2000; Gajdocsy and Horvath, 2010). Currently, an increased eCO level has been implicated as a possible indicator of pulmonary or systemic inflammation, and the measurement of eCO levels has been studied as a useful inflammatory marker of respiratory diseases such as asthma and interstitial lung disease.

**Examples of Increased eCO Production in Chronic Lung Diseases**

**Asthma**

Asthma, an obstructive lung disease, is characterized by chronic inflammation and recurrent exacerbations resulting airway obstruction that can be reversible after a pharmacological intervention, such as administration of bronchodilators. Increased production of eCO in asthmatics has been confirmed by many studies (Zayasu et al, 1997; Horvath et al, 1998b; Wood et al, 2003; Gajdocsy and Horvath, 2010), and the results suggest that expression

of HO-1 in epithelial cells of the airway is increased in airway inflammation. It has also been shown that eCO levels are further increased during acute asthma and are usually decreased or within the normal range in patients who are stable on inhaled corticosteroids (Zayasu et al, 1997; Horvath et al, 1998b). The improvement of pulmonary functions during asthma therapy is accompanied by eCO decline and a decline in eosinophil count in sputum (Yamaya, 1999).

**Chronic obstructive pulmonary disease (COPD)**

COPD is also an obstructive lung disease and characterized by airflow obstruction with breathing-related symptoms such as chronic cough, increased mucus production, and dyspnea on exertion (Rennard, 1998). Patients with COPD have elevated levels of eCO, and current smokers with COPD had higher eCO levels than ex-smokers with COPD (Montuschi et al, 2001; Kharitov et al, 2002). The increased levels of eCO in COPD patients suggest that HO-1 expression can be induced by cigarette smoke. There is an increase in exhaled CO during acute exacerbations of COPD, with a decline after recovery (Biernacki, 1999). However, there is a limitation in using eCO as a marker of COPD since eCO is elevated in COPD patients and also in normal smokers (Montuschi et al, 2001).

**Interstitial lung diseases (ILD)**

Interstitial lung disease (ILD) is a restrictive lung disease and refers to a broad group of inflammatory lung disorders such as pulmonary fibrosis, asbestosis, or scleroderma that can result in interstitial thickening, fibrosis, or granulomas. ILD is distinguished by the presence of chronic alveolitis that produces a derangement of the alveolar structures and ultimately leads to loss of functional gas exchange units with resultant disordering of the lung's connective tissue skeleton (“fibrosis”) and injury of the parenchymal cell populations (Crystal et al., 1981). Elevation of eCO has been observed in patients with ILD related to lung function deterioration

with a failure of gas transport (Antuni et al, 1999). Elevated levels of eCO in patients with ILD are also associated with disease progression (Babusikova et al., 2008), and thus the results suggest that eCO can serve as a diagnostic tool to monitor interstitial lung disease progression and the response to therapy.

# CHAPTER II: INTRODUCTION

## Statement of the Research Problem

Optimizing the utilization of pulmonary function test (PFT), by primary care physicians, is a critical component of diagnosis and an integral part of monitoring effective treatments for respiratory problems. However, PFT is not well utilized by primary care physicians in spite of the clinical importance of PFT. This is primarily due to a lack of well-established indicators for PFT in the diagnosis of patients with chronic lung diseases.

To address this shortcoming, there is a need to establish a standardized indicator for PFT to help primary care physicians make a diagnosis and offer effective treatments to patients. Some studies have reported that exhaled carbon monoxide (eCO), which is used as an indicator of smoking, is significantly increased in patients with chronic lung diseases such as asthma and not being treated with corticosteroids (Zayasu et al., 1997; Horvath, 1998b; Kharitonov, 2004; Zhang, 2010). All organisms having heme produce carbon monoxide (CO) endogenously, and CO in the body is mainly derived from the degradation of hemoglobin by the enzyme heme oxygenase (HO) (Kharitonov & Barnes, 2002). When cells are under oxidative stress, the production of carbon monoxide (CO) is increased in the body as a breakdown product of heme by the stress-induced isoform of heme oxygenase, HO-1 (Paredi et al., 2002). These findings suggest that levels of eCO, which can be simply measured in expired air, might be useful as a non-invasive biomarker for assessing airway inflammation, the prominent characteristic for chronic lung diseases such as asthma and COPD. Figure 2 summarizes the pathophysiology for increased eCO and abnormal PFT by lung inflammation.

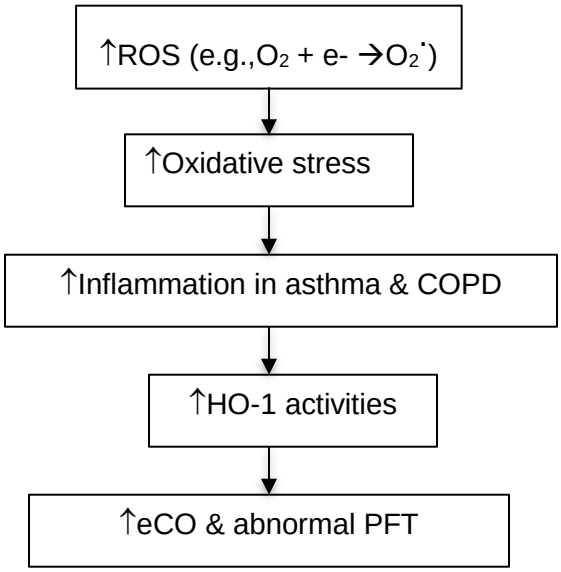



Figure 2. Pathophysiology for increased eCO and abnormal PFT by lung inflammation.

**Hypotheses**

Obstructive lung diseases occur when something obstructs the flow of air into or out from the lungs and are characterized by airflow limitation. Spirometry measures the volume of air exhaled and inhaled by a patient as a function of time, and the American Thoracic Society (ATS) defines airflow obstruction as a reduction in the ratio of forced expiratory volume in 1 second ($FEV_1$) to forced vital capacity (FVC) (Pellegrino et al., 2005). Therefore, reductions in the FEV1/FVC ratio less than 70% with a presence of reduced FEV1, which is less than 80%, are typical obstructive disorders, implying airway narrowing during exhalation. The FEV1 is used to grade the severity of obstruction. The earliest change associated with airflow obstruction usually occurs in small airways, less than 2 mm in diameter, and it may be detected by $FEF_{25-75\%}$, the mean expiratory flow rate in the middle half of the FVC maneuver (Hankinson et al., 1999). An obstructive defect is also indicated by increased levels of Functional Residual Capacity (FRC) due to air-trapping and decreased levels of diffusing capacity of lungs for carbon monoxide (DLCO) due to the loss of alveolar surface area.

Restrictive lung diseases occur when one cannot inhale a normal amount of air and are characterized by a reduction of lung volumes. A restrictive defect is indicated by decreased levels of FVC, FEV1, and total lung capacity (TLC). DLCO values also decrease due to the inflammation of alveoli. Therefore, the hypotheses are as follows:

**Hypothesis #1:** Patients with obstructive lung diseases such as asthma, COPD, or cystic fibrosis will demonstrate

- **a.** increased levels of eCO with decreased levels of Forced Expiratory Volume (FEV1).
- **b.** increased levels of eCO with decreased levels of Forced Expiratory Flow ($FEF_{25-75\%}$).
- **c.** increased levels of eCO with increased levels of Functional Residual Capacity (FRC).
- **d.** increased levels of eCO with decreased levels of diffusing capacity of lungs for carbon monoxide (DLCO).

**Hypothesis #2:** Patients with restrictive lung diseases such as pulmonary fibrosis or pneumonia will demonstrate

- **a.** increased levels of eCO with decreased levels of Forced Vital Capacity (FVC).
- **b.** increased levels of eCO with decreased levels of Forced Expiratory Volume (FEV1).
- **c.** increased levels of eCO with decreased levels of Total Lung Capacity (TLC).
- **d.** increased levels of eCO with decreased levels of diffusing capacity of lungs for carbon monoxide (DLCO).

The purpose of this research is to evaluate endogenous carbon monoxide concentration in the exhaled breath of patients with lung diseases that involve lung inflammation and compare the results with PFT measurements to establish a standardized indicator for PFT.

## Significance of Study

Pulmonary function testing typically involves measurement of the lung's physical and functional properties, such as lung mechanics, airflow, and gas exchange, allowing clinicians to evaluate lung pathophysiology. However, patients are often sent to a pulmonary function testing lab too late to alter the dysfunction of respiratory diseases, thus delaying treatments (Chapman and Choi, 2001). Recent studies, however, have shown that exhaled carbon monoxide gas (eCO) may contain valuable molecular clues to lung cell function (Horvath, 1998a).

A diagnostic method that will render accuracy in a minimal amount of time is the ultimate goal of most clinicians. If the diagnosis of lung disease can be achieved quickly and effectively with measuring eCO, early therapeutic interventions will be possible and patients would spend less time in an inpatient setting and reduce overall healthcare costs.

This study seeks to determine whether eCO measurement can be utilized as an indicator for conducting PFT. This information may be extremely beneficial for the participants directly involved in the study and for clinicians looking for a standardized indicator for conducting PFT to determine lung pathophysiology that allows early intervention with drug therapy and as well as monitoring the effects of the drugs. The potential benefit to be gained by persons participating in this study is to receive free eCO evaluation added to the standard PFT already being done as part of their medical diagnostic tests.

The information gained through this study may improve diagnostic procedures used by clinicians and benefit patients with chronic lung diseases since monitoring of eCO concentrations can be a simple, non-invasive, cost-effective, and reproducible method to evaluate the progression and severity of oxidative stress and inflammation. This knowledge will be extremely useful in defining indications for PFT, diagnosing chronic lung diseases such as asthma and COPD, identifying effective treatment strategies, and monitoring the outcomes of steroid drug treatments.

**Definition of Terms**

1. **eCO** = Exhaled Carbon Monoxide
2. **Spirometry** =A test to assess pulmonary mechanics by measuring lung volumes and flows.
3. **FVC (Forced Vital Capacity)** = The largest volume of air that can be forcefully expired from the lungs.

4. **$FEV_1$ (Forced Expiratory Volume)** = The largest volume that can be forcefully expired in the first second.
5. **$FEV_1/FVC$** = A ratio which represents the proportion of forced vital capacity that is exhaled in the first second of forced expiration.
6. **$FEF_{25-75\%}$ (Forced Expiratory Flow)** = The average flow measured over the middle 50% of an FVC maneuver.
7. **DLCO** (Diffusing Capacity of Lung for Carbon Monoxide) = A test to measure the transfer of a diffusible gas, which is carbon monoxide (CO), across the alveolar-capillary membrane.
8. **TLC (Total Lung Capacity)** = The volume of gas contained in the lungs after maximal inspiration (Fig. 3).
9. **FRC (Functional Residual Capacity)** = The volume of gas remaining in the lungs at the end of resting breathing.
10. **RV (Residual Volume)** = The volume of gas remaining in the lungs at the end of a maximal expiration.
11. **VC (Vital Capacity)** = The largest volume of air that can be expired from the lungs.

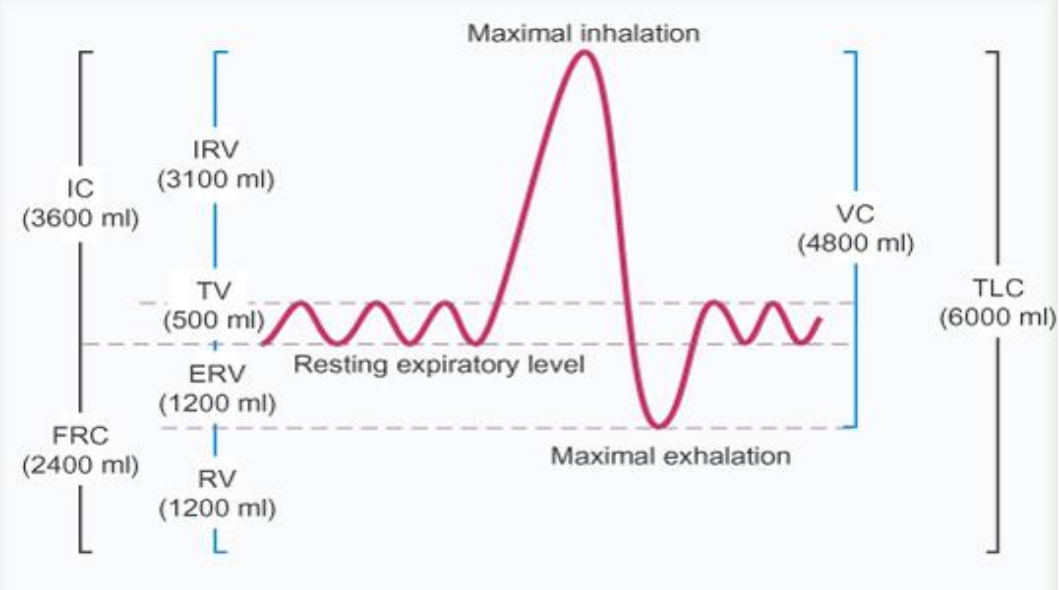



Figure 3. Lung volumes and capacities (Kacmarek et al., 2017).

# CHAPTER III: METHODOLOGY

## Experimental Design

This research is a cross-sectional descriptive study that evaluates the use of a carbon monoxide analyzer as a diagnostic test for eCO and as a potential indicator for a referral to pulmonary function test (PFT). This study was approved by the Queen's Medical Center Institutional Review Committee (See Appendix A). After consenting, the subjects' eCO levels were measured with a portable carbon monoxide analyzer from a single exhalation. If the subjects were smokers, their smoking histories were documented (as part of a study health questionnaire). Volume and flow calibrations of PFT equipment were done daily with a calibrated 3L syringe during the study period. After data collection and initial analysis, subjects were divided into three groups based on their diagnosis: (1) obstructive, (2) restrictive, and (3) signs and symptoms only. Each group was further divided into two sub-groups: untreated and treated with an anti-inflammatory drug.

## Human Subject Interactions

Research subjects were recruited from the Queen's Medical Center Pulmonary Lab after physician referral for a routine care PFT. Prospective study participants received a study information sheet as part of the informed consent process and the study was explained by the P.I. or respiratory therapists. All questions were answered to the subject's satisfaction.

Following consent, an initial health questionnaire was given to the consented individuals, and clarification was provided if necessary. The questionnaire was anonymized and did not include identifying information, such as name or medical record number, except for the test date, age, and a study-specific ID to track the recruitment process. The date information was used to correlate weather conditions (like vog or temperature) on the day of testing to the test results.

Age, gender, height, weight, ethnicity, and body type (normal or muscular) were included in the analysis of the results. The questionnaire data were collected in an envelope and transcribed into a computer database on a weekly basis.

**Eligibility Criteria**

**Inclusion criteria**

- Persons who had symptoms of acute or chronic lung diseases such as asthma and COPD and were referred to the Pulmonary Lab at the Queen's Medical Center by their physicians for PFT.
- Subjects who were 18 years or older.
- Control subjects were included, who did not have symptoms of acute or chronic lung disease.

**Exclusion criteria**

- Inpatients
- Outpatients who were too sick to perform the testing maneuvers.
- Subjects who were unable to follow study procedures.

**Recruitment /Enrollment**

Subjects with a pulmonary disease were recruited for this study by the investigators and other research personnel through direct contact with patients who had been referred to the QMC Pulmonary Lab for routine care by their physicians. In addition, a control group was enrolled, who were non-pulmonary patients and healthy. If someone from the control group uses an anti-inflammatory drug, he or she was excluded.

A study-specific information sheet was provided to the subjects as part of the informed consent process. The study purpose, its benefits and risks, study-related procedures and the voluntary nature of participation were explained (see Recruitment Script, appendix D). All

questions were answered to the patient's satisfaction before consenting and before any study related activity occurred.

**Subject withdrawal criteria**

Subjects were allowed to withdraw from the study at any point and for any reason. Subjects were withdrawn from the study if they failed to follow directions of the research staff, or if medically indicated.

## Study Procedures

### Study Questionnaire

Following the consent process, research subjects were given a health questionnaire by the investigators or other study personnel. On the questionnaire, the subjects were asked to report on their well-being and symptoms at the time of referral, current and past smoking habits, eating habits, their diagnosis (if known) and use of inhaler medication for their lung disease. The questionnaires contained a study-specific ID (to track the progress of recruitment) but were otherwise largely anonymized: collecting age (in years) and the PFT test date. All individual data were kept strictly confidential (not shared outside the study). The backside of the questionnaire served as a data collection sheet (see Appendix D) for the eCO measurement and selected PFT data.

### Carbon Monoxide Analyzer (study procedure)

Exhaled carbon monoxide (eCO) levels were measured with a portable carbon monoxide analyzer (Model: MicroCO Meter, CareFusion, Yorba Linda, CA) from a single exhalation. The subjects were asked to exhale fully, inhale deeply and hold breath for 8-10 seconds (to let any

inhaled environmental CO be taken away by blood and endogenously produced CO to equilibrate) and exhale through a disposable mouthpiece into the CO analyzer.

The measurement was done before the subjects performed PFTs to avoid interference from the lung function test, such as Diffusing Capacity of Lung for Carbon Monoxide (DLCO) test, in which patients inhaled carbon monoxide during the test.

**Pulmonary Function Test (routine care)**

Subjects had PFT as their scheduled routine procedure, ordered by their physician. The PFT was performed by one of the certified research team members who recorded the eCO levels and the PFT results into a data collection form, thereby linking the subjects' information without the need of any identifiers. No identifying information was transcribed, and no access to the subject's medical record was required. Recruitment process and study procedures are shown in Fig. 4.

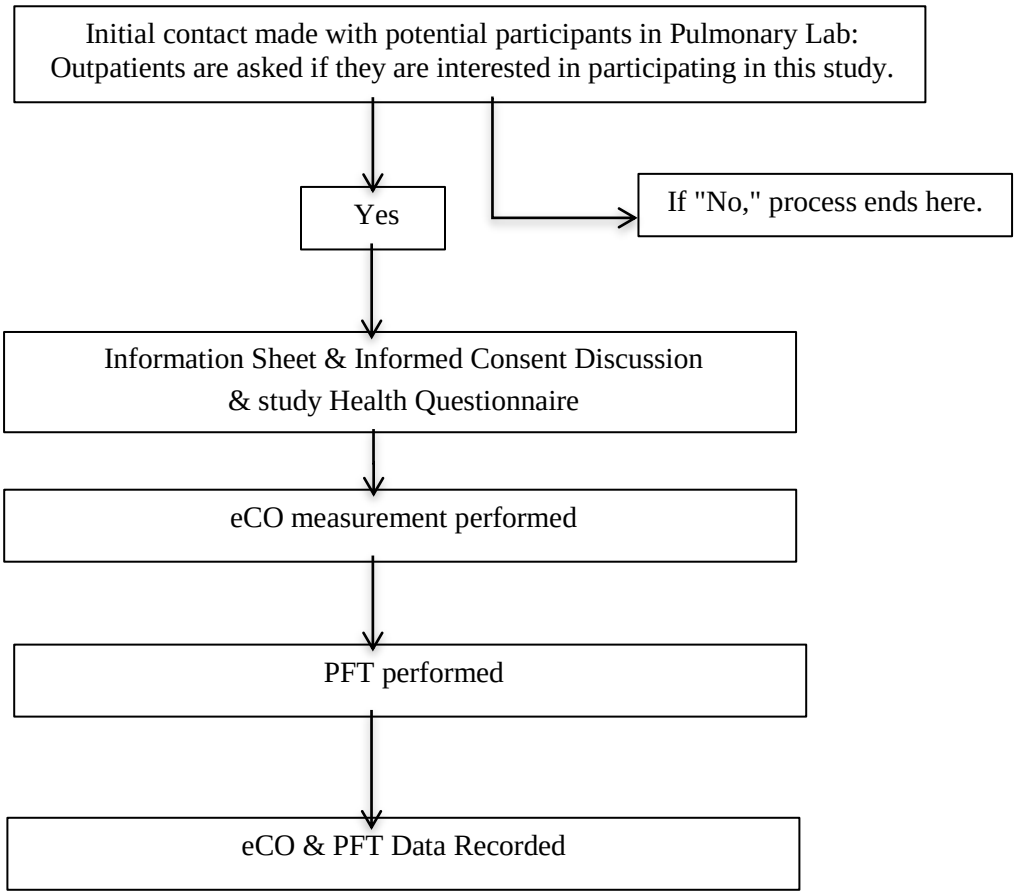



Figure 4. Recruitment and study process.

PFT results were expressed as percentages of the predicted values and were calculated using Crapo's reference standards (2005). The standards were based on a cohort of normal subjects of similar age, height, and race, with normal being defined as persons without a history of smoking or disease that can affect lung function. PFTs were performed using the VMAX (Model: Encore 229) and JAEGER (CareFusion, Yorba Linda, CA).

**Data Analysis**

Analyses were performed using JMP software, version Pro 14.0, (SAS Institute, Cary, North Carolina, USA) for exploratory data analysis. Data analysis was performed to explore variable distributions and correlations and to examine how the abnormal eCO values affect the

sensitivity and specificity of predicting commonly used diagnostic measures in PFT. Variables were summarized with descriptive statistics (n, mean, standard deviation, and median). Statistical analysis was performed by one-way analysis of variance, and significance was accepted at a p-value less than or equal to 0.05.

# CHAPTER IV: RESULTS

## Sample Characteristics

A total of 366 subjects (344 patients and 22 control subjects) underwent exhaled carbon monoxide (eCO) measurements and Pulmonary Function Testing (PFT) during the study period. After a review of the data, one subject from the patient's group was excluded from the study due to erroneously recorded data. Another subject from the control group was excluded due to corticosteroid drug use, making the total number of subjects 364 (343 patients and 21 control subjects).

The descriptive characteristics of the remaining entire sample (n=364) are presented in Table 1. Age ranged from 18 to 92 years, with a mean of 62.5 (SD=14.2) years. One hundred eighty-six participants (55.5%) were female, and one hundred fifty-seven (44.5%) were male. One hundred thirty-seven participants (39.9%) were diagnosed with obstructive lung disease, fifty participants (14.6%) were diagnosed with restrictive lung disease, and one hundred fifty-six participants (45.5%) were diagnosed with signs and symptoms such as shortness of breath or dyspnea. The control group consisted of 16 females, age ranged from 21 to 40 years of age with a mean of 27.6 (SD=6.2) years, and 5 males, age ranged from 34 to 52 years with a mean of 35.0 (SD=11.4) years. The mean BMI was 24.4 $kg/m^2$ in females and 27.4 $kg/m^2$ in males.

Table 1. Participants Characteristics

| | Patient Group | | Control Group | |
|---|---|---|---|---|
| | Female | Male | Female | Male |
| N | 186 (55.5%) | 157 (44.5%) | 16 (71.6%) | 5 (28.4%) |
| Age | 62.5 yrs | 59.5 yrs | 27.6 yrs | 35.0 yrs |
| Ethnicity | | | | |
| Arabian | 0 | 1 (0.3%) | 0 | 0 |
| Asian | 79 (23.1%) | 53 (15.5%) | 8 (38.1%) | 3 (14.3%) |
| Black | 2 (0.6%) | 4 (1.2%) | 0 | 0 |
| Caucasian | 48 (14.0%) | 40 (11.7%) | 2 (9.5%) | 0 |
| Eurasian | 4 (1.2%) | 5 (1.5%) | 0 | 1 (4.8%) |
| Filipino | 23 (6.7%) | 27 (7.9%) | 5 (23.8%) | 0 |
| Micronesian | 1 (0.3%) | 5 (1.5%) | 0 | 0 |
| Part-Hawaiian | 23 (6.7%) | 18 (5.3%) | 0 | 1 (4.8%) |
| Polynesian | 5 (1.5%) | 3 (0.9%) | 0 | 0 |
| Vietnamese | 0 | 1 (0.3%) | 1 (4.8%) | 0 |
| Smoke | | | | |
| Yes | 16 (4.7%) | 27(7.9%) | 1 (4.8%) | 1 (4.8%) |
| No | 167 (49.1%) | 130 (38.2%) | 15 (71.4%) | 4 (19.0%) |
| Quit | 58 | 72 | 2 | 2 |
| Body Mass Index | 27.5 $kg/m^2$ | 29.7 $kg/m^2$ | 24.4 $kg/m^2$ | 27.4 $kg/m^2$ |
| Anemia | | | | |
| Yes | 33 (9.7%) | 19 (5.6%) | 3 (14.3%) | 0 |
| No | 152 (44.6%) | 137 (40.2%) | 15 (61.9%) | 4 (19.0%) |
| Anti-inflammatory Drugs | | | | |
| Yes | 53 (15.5%) | 34 (9.9%) | 0 | 0 |
| No | 133 (38.8%) | 123 (35.9%) | 0 | 0 |
| Consumption of Meats/week | | | | |
| Beef | 1.9 | 2.3 | 2.0 | 2.6 |
| Pork | 1.4 | 1.7 | 1.4 | 1.2 |
| Poultry | 2.6 | 3.0 | 2.2 | 3.2 |
| Fish | 2.0 | 2.3 | 1.6 | 2.0 |

*Diagnosis*

The diagnosis of obstructive lung disease included COPD, asthma, bronchitis, bronchiolitis, bronchiectasis, emphysema, and alpha-1 antitrypsin deficiency. The diagnosis of restrictive lung disease included pulmonary fibrosis, atelectasis, interstitial lung disease, pleural effusion, pneumonia, pneumonitis, pulmonary nodule, and diseases that would result in restrictive lung diseases such as connective tissue disease, Crest syndrome, Hodgkin's

lymphoma, scleroderma, and systemic lupus erythematosus. The diagnosis of signs and symptoms included chronic or acute cough, chest tightness or pain, shortness of breath, dyspnea on exertion or resting, fatigue, hypoxemia, wheezing, and abnormalities on chest x-rays such as lung mass or nodule. The major symptoms for referral were shortness of breath (n=44, 28.2%), chronic cough (n=25, 16.0%), dyspnea (n=25, 16.0%), and dyspnea on exertion (n=14, 9.0%). Shortness of breath as a major symptom was more common in female (n=28) than male (n=16) patients and in the age group of 50 to 79 years (n=32).

*Smoking History and Body Mass Index*

Sixteen (4.7%) females and twenty-seven (7.9%) males were current smokers. The majority of the patients were overweight or obese with body mass index (BMI) being over 25 $kg/m^2$, 99 (53%) for females and 115 (73%) males. Most patients were not anemic; 33 (9.7%) females and 19 (5.6%) males reported to have been anemic. The mean hemoglobin count of the participants was 13.1 mg/dL for females and 14.2 mg/dL for males.

*Drug Use*

Eighty-seven participants (25.4%) reported the use of anti-inflammatory drugs (Table 1), and the most commonly used drug was Advair® (n=29, 14 females and 5 males), a combination of fluticasone (corticosteroid) and salmeterol (long-acting bronchodilator). Two hundred ninety-four (79.1%) participants were using at least one drug not only for lung diseases but also for other diseases, and the maximum number of the drugs used by the participants was 15 (Table 2).

*Diet*

The dietary habits of the participants were recorded to find out iron deficiency (Table 1). Females reported a mean consumption of beef, pork, poultry, and fish as 1.9, 1.4, 2.6, and 2.0 per

week, respectively, and males as 2.3, 1.7, 3.0, and 2.3 per week, respectively. Only 1 female and male were vegetarian, and none of them were reported as a vegan.

Table 2. Number of Drugs Used by the Participants

| Number of Drugs | Number of Participants (N) | % of Total |
|---|---|---|
| 0 | 50 | 15.3% |
| 1 | 61 | 17.5% |
| 2 | 63 | 19.3% |
| 3 | 34 | 10.4% |
| 4 | 34 | 10.4% |
| 5 | 28 | 8.6% |
| 6 | 17 | 5.2% |
| 7 | 17 | 5.2% |
| 8 | 6 | 1.8% |
| 9 | 8 | 2.4% |
| 10 | 7 | 2.1% |
| 13 | 1 | 0.3% |
| 14 | 1 | 0.3% |
| 15 | 1 | 0.3% |

## Pulmonary Function Test (PFT) and eCO Test Results

### Pulmonary Function Test (PFT)

PFT results of all groups are shown in Table 3 (female subjects) and Table 4 (male subjects). Based on the American Thoracic Society (ATS) Guidelines (Pellegrino et al., 2005), an obstructive defect is indicated by FVC, $FEV_1$, and $FEF_{25-75\%}$ less than 80%, and $FEV_1$/FVC less than 70% of the predicted normal values. A restrictive defect is indicated by a total lung capacity (TLC) less than 80%. DLCO values decrease, less than 80% of the predicted normal values, for both obstructive and restrictive lung disease.

Spirometry results of the control group were normal since FVC, $FEV_1$, $FEF_{25-75\%}$, and $FEV_1$/FVC were greater than 80% of the predicted normal values in both females and males. Lung volume test results were also normal; total lung capacity (TLC) and functional residual capacity (FRC) being within normal limits. However, DLCO test results revealed that females in the control group had a mild diffusion defect, 20.4 L/s/mm Hg or 68% of the predicted normal values, which can happen to healthy individuals, while males produced normal DLCO results, 82% with a mean of 31.8 L/s/mm Hg.

Table 3. Exhaled CO Levels and PFT Results of Female Participants

| **Tests and Characteristics of participants** | **Control Group (N=16)** | **Subjects with Obstructive Lung Disease (N=75)** | **Subjects with Restrictive Lung Disease (N=29)** | **Subjects with Signs &Symptoms (N=82)** |
|---|---|---|---|---|
| **Exhaled CO** (ppm) | 4.1±0.6 | 10.3±0.9[c] | 8.4±0.9[b] | 9.4±1.2[b] |
| **Spirometry** | | | | |
| FVC, L (% Ref) | 3.3[1] (92±2.3)[2] | 2.4 (87±2.0) | 2.7 (94±4.4) | 2.5 (88±2.0) |
| FEV1, L (% Ref) | 3.0 (92±2.4) | 1.8 (80±2.5)[a] | 2.1 (93±4.5) | 2.0 (87±2.0) |
| $FEF_{25-75\%}$, L/sec (%Ref) | 3.6 (94±.41) | 1.4 (65±4.1)[b] | 1.9 (88±7.4) | 1.7 (75±2.7)[c] |
| FEV1/FVC, % | 89±1.2 | 72±1.4[d] | 78±1.2[d] | 78±0.7[d] |
| **Lung Volume** | | | | |
| TLC, L (% Ref) | 3.6 (94±1.9) | 4.2 (88±1.7) | 4.2 (87±3.7) | 4.2 (87±1.4) |
| FRC, L (% Ref) | 2.5 (92±3.6) | 2.8 (90±2.2) | 2.3 (85±3.8) | 2.4 (88±1.7) |
| RV, L (% Ref) | 1.1 (87±5.4) | 1.7 (87±2.8) | 1.5 (80±4.6) | 3.6 (83±2.4) |
| **Diffusion (DLCO)** | | | | |
| L/s/mm Hg (%Ref) | 20.4 (68±2.0) | 14.7 (63±1.9) | 13.8 (57±3.0)[a] | 15.4 (64±1.5) |
| Hb, g/dL | | 13.1 | 12.7 | 13.1 |
| **Age** (Years) | 27.6±3.4 | 63.9±1.6 | 62.1±2.6 | 61.5±1.5 |
| **BMI** ($kg/m^2$) | 24.4±2.1 | 27.7±0.9 | 25.3±1.6 | 27.9±0.9 |

[1]Expressed as mean of actual values in spirometry, lung volume, and DLCO. [2]( ) indicates percentage of actual values expressed as mean ± SEM against reference values in spirometry, lung volume, and DLCO. Superscripts on PFT results indicate levels of statistical significance for comparisons of values within rows: [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.005$; [d]$P < 0.0001$; no superscript, $P > 0.05$ based on one-way ANOVA.

Table 4. Exhaled CO Levels and PFT Results of Male Participants

| Tests and Characteristics of participants | Control Group (N=5) | Subjects with Obstructive Lung Disease (N=62) | Subjects with Restrictive Lung Disease (N=21) | Subjects with Signs &Symptoms (N=74) |
|---|---|---|---|---|
| **Exhaled CO** (ppm) | 5.0 ±1.2 | 15.3±1.8 [a] | 12.3±2.5 [a] | 12.1±1.2 [a] |
| **Spirometry** | | | | |
| FVC, L (% Ref) | 5.1[1] (96±5.7)[2] | 3.3 (77±2.2)[a] | 3.2 (80±3.8) | 3.8 (85±2.0) |
| FEV1, L (% Ref) | 4.4 (102±7.2) | 2.3 (66±2.8)[c] | 2.3 (73±4.7)[a] | 2.9 (81±2.2)[a] |
| $FEF_{25-75\%}$, L/sec (%Ref) | 5.4 (119±16.8) | 1.6 (49±4.2)[b] | 1.8 (54±5.8) [c] | 3.5 (72±3.2)[b] |
| FEV1/FVC, % | 87±3.3 | 67±1.7[c] | 70±2.4[c] | 76±1.0[c] |
| **Lung Volume** | | | | |
| TLC, L (% Ref) | 6.4 (93±4.6) | 5.8 (90±2.1) | 5.4 (84±2.9) | 6.0 (92±1.9) |
| FRC, L (% Ref) | 3.3 (98±7.1) | 3.4 (101±3.1) | 3.2 (95±3.6) | 3.3 (99±2.3) |
| RV, L (% Ref) | 1.4 (76±16.4) | 2.4 (112±4.5) | 2.1 (92±6.3) | 2.2 (105±3.3) |
| **Diffusion (DLCO)** | | | | |
| L/s/mm Hg (%Ref) | 31.8 (82±4.3) | 18.9 (60±2.5)[a] | 15.9 (55±2.7)[c] | 20.7 (64±1.7)[b] |
| Hb, g/dL | | 14.4 | 12.1 | 14.5 |
| **Age** (Years) | 35.0±6.4 | 60.4±1.8 | 68.6±3.1 | 56.9±1.7 |
| **BMI** ($kg/m^2$) | 27.4±2.9 | 30.5±0.8 | 28.7±1.4 | 29.4±0.8 |

[1]Expressed as mean of actual values in spirometry, lung volume, and DLCO. [2]( ) indicates percentage of actual values expressed as mean ± SEM against reference values in spirometry, lung volume, and DLCO. Superscripts on PFT results indicate levels of statistical significance for comparisons of values within rows: [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.005$; [d]$P < 0.0001$; no superscript, $P > 0.05$ based on one-way ANOVA.

*Obstructive Lung Disease Group*

The PFT results of females of the obstructive disease group indicated a mild airflow limitation and diffusion defect with a mean $FEF_{25-75\%}$ of 1.4 L/s or 65% (p=0.0005) and a mean DLCO of 14.7 L/s/mm Hg or 63% (p=0.3125) of the predicted normal values (Table 3). These results are consistent with the diagnosis of obstructive lung disease. FVC values were not significantly different between female groups; however, $FEV_1$, $FEF_{25-75\%}$, $FEV_1$/FVC were significantly lower in the obstructive lung disease group (Figure 5).

Males in the obstructive disease group also produced a mild to moderate airflow limitation and diffusion defect, as shown in Table 4, with a mean $FEV_1$ of 2.3 L (66%,

p=0.0023), $FEF_{25-75\%}$ of 1.6 L (48%, p=0.0014), $FEV_1$/FVC of 67% (p=0.0050) and DLCO of 18.9 (60%, p=0.0102). The spirometry values of the male group were also significantly lower in the obstructive lung disease group as shown in Figure 6.

*Restrictive Lung Disease Group*

Female subjects with the restrictive disease diagnosis produced normal spirometry results since FVC, $FEV_1$, and $FEF_{25-75\%}$ values were greater than 80%, and $FEV_1$/FVC value was greater than 70% of the predicted normal values, an indication of no airflow limitation (Table 3). $FEV_1$ and $FEF_{25-75\%}$ values of this group were significantly higher than those of other groups (Figure 5). Their lung volume tests also produced normal values, as shown in Table 2, TLC of 4.2 L (87%) and FRC of 2.3 L (84%). However, their DLCO results showed a moderate diffusion defect with a mean DLCO of 13.8 L/s/mm Hg (57%, p=0.0083).

PFT results of the male subjects in the restrictive disease group, on the other hand, revealed both a mild to moderate airflow limitation and diffusion defect, as shown Table 4, with a mean $FEV_1$ of 2.4 L (76%, p=0.0287), $FEF_{25-75\%}$ of 1.8 L (58%, p=0.0044), and DLCO of 15.9 (55%, p=0.0091). All of the spirometry values, FVC, $FEV_1$, $FEF_{25-75\%}$, and $FEV_1$/FVC, of males in the restrictive lung disease group, are higher than the obstructive lung disease group but lower than the signs and symptoms group (Figure 6). All of the parameters in lung volumes test, TLC, FRC, and RV, were significantly lower in both females and males in the restrictive lung disease group (Figure 7 & 8). DLCO values were also significantly lower in both males and females in the restrictive lung disease group (Figure 9).

*Signs and Symptoms Group*

In the group diagnosed with signs and symptoms, female subjects displayed a mild airflow limitation and diffusion defect with a mean $FEF_{25-75\%}$ of 1.7 L/s (75%, p=0.0022) and

DLCO of 15.4 L/s/mm Hg (64%, p=0.3431). Male subjects also reported a mild airflow limitation and diffusion defect with a mean $FEV_1$ of 2.9 L (80%, p=0.0136), $FEF_{25-75\%}$ of 3.5 L/s (72%, p=0.0069) and a mean DLCO of 20.7 L/s/mm Hg (64%, p=0.0067). The spirometry values of males in the signs and symptoms group were significantly higher than the other two patient groups (Fig. 5).

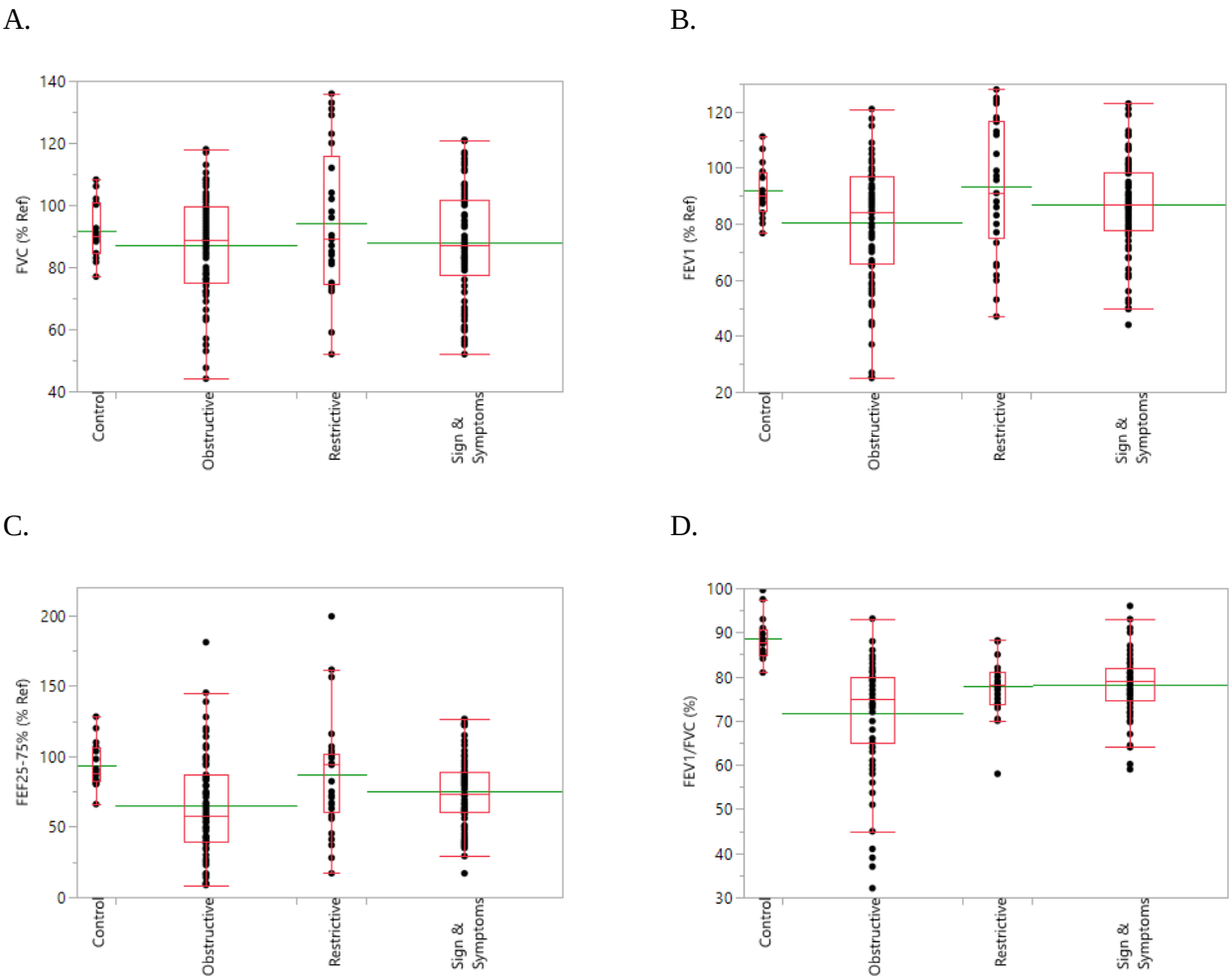
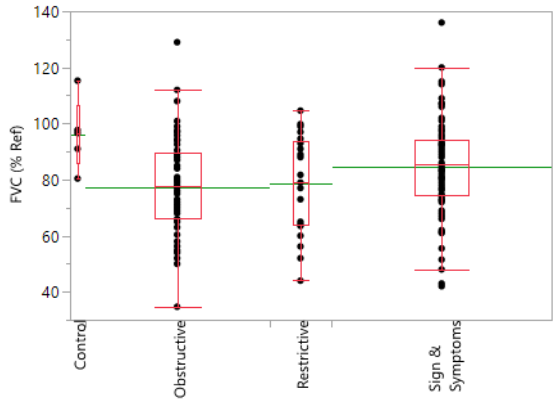



Figure 5. Female spirometry results for Control, Obstructive, Restrictive, and Signs & Symptoms group.
* The green horizontal lines crossing the boxes represent the mean values. The red horizontal lines within the boxes represent the median values. The ends of the boxes represent the 25th and 75th quantiles. The whiskers represent 1.5 x interquartile ranges from the boxes. The disconnected points are potential outliers.

A.

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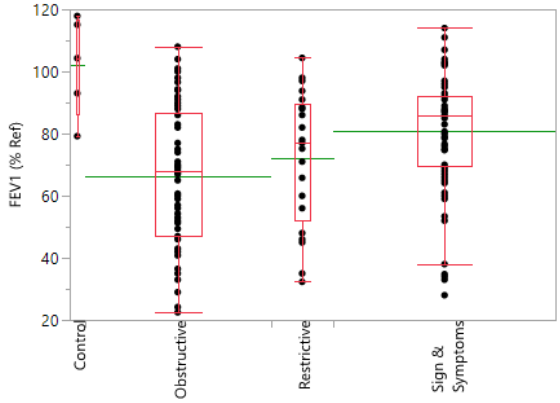
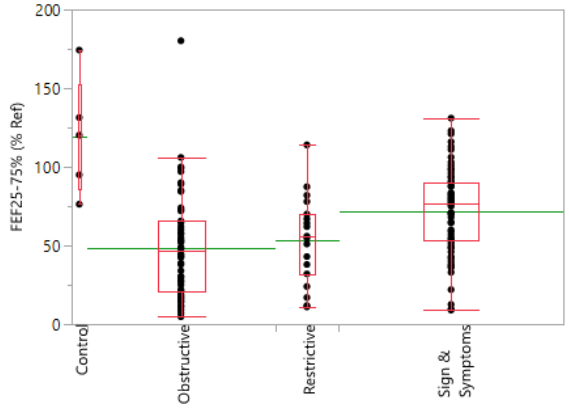



C.




D.

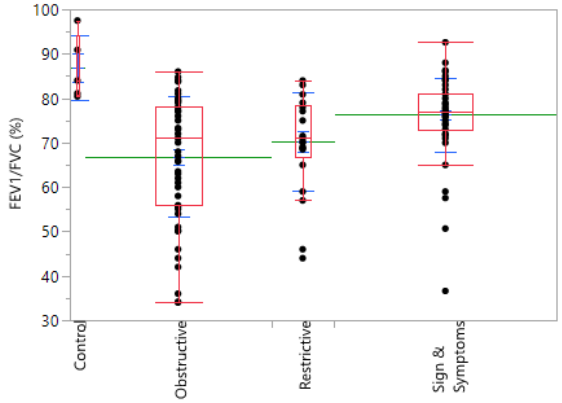
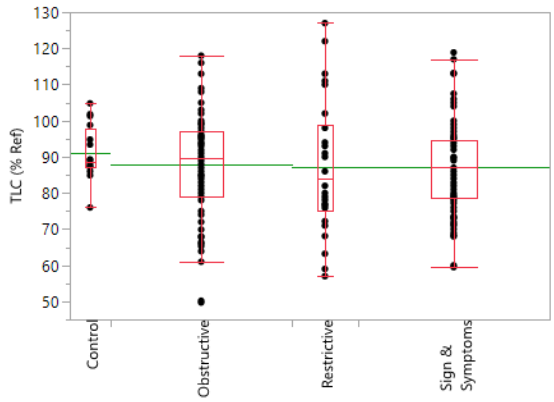



Figure 6. Male spirometry test results for Control, Obstructive, Restrictive, and Signs & Symptoms group.

* The green horizontal lines crossing the boxes represent the mean values. The red horizontal lines within the boxes represent the median values. The ends of the boxes represent the 25th and 75th quantiles. The whiskers represent 1.5 x interquartile ranges from the boxes. The disconnected points are potential outliers.

A.

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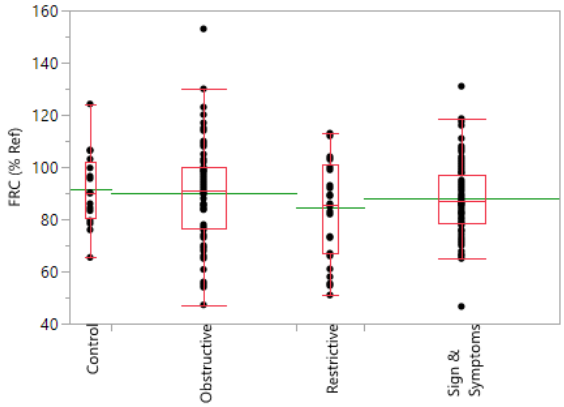


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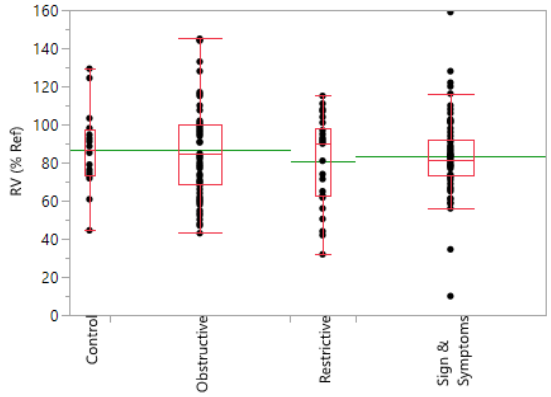
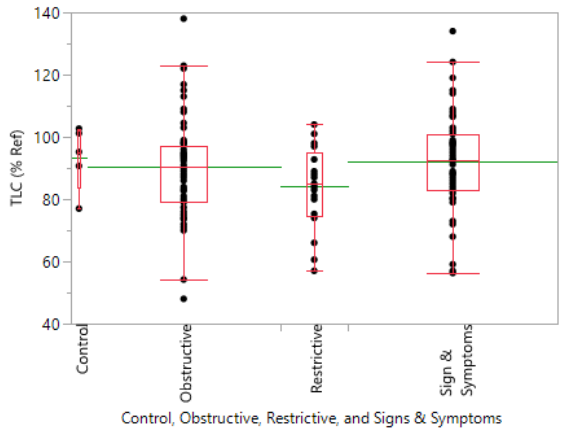


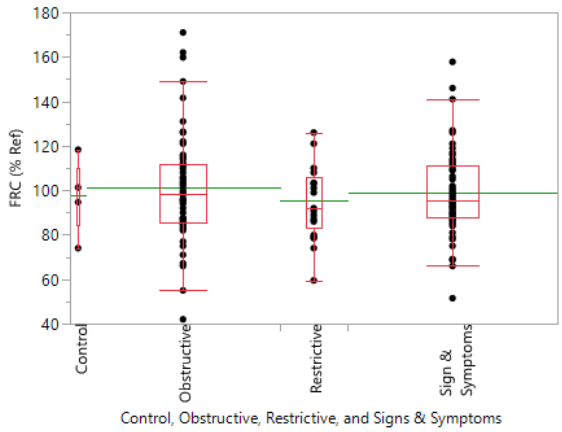

Figure 7. Female lung volume test results for Control, Obstructive, Restrictive, and Signs & Symptoms group.

* The green horizontal lines crossing the boxes represent the mean values. The red horizontal lines within the boxes represent the median values. The ends of the boxes represent the 25th and 75th quantiles. The whiskers represent 1.5 x interquartile ranges from the boxes. The disconnected points are potential outliers.

A.




B.




C.

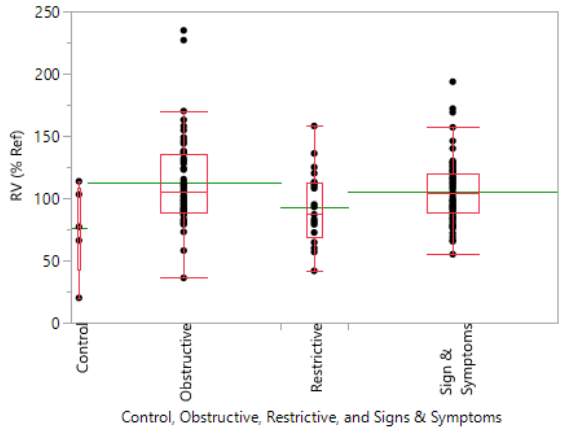
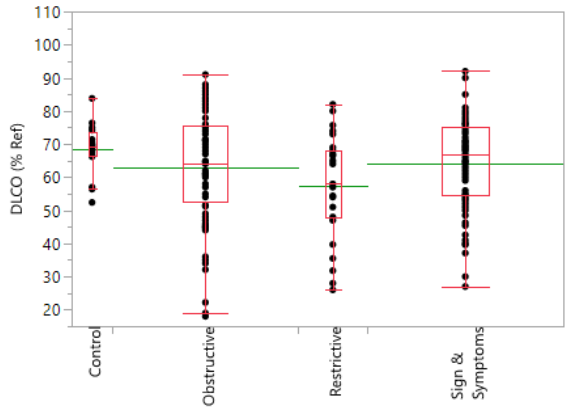



Figure 8. Male lung volume test results for Control, Obstructive, Restrictive, and Signs & Symptoms group.

* The green horizontal lines crossing the boxes represent the mean values. The red horizontal lines within the boxes represent the median values. The ends of the boxes represent the 25th and 75th quantiles. The whiskers represent 1.5 x interquartile ranges from the boxes. The disconnected points are potential outliers.

A. Female Group




B. Male group

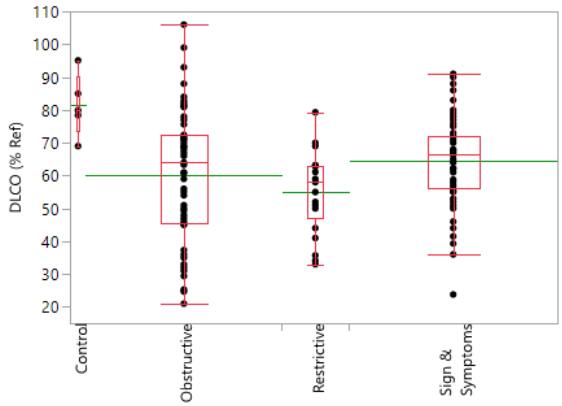



Figure 9. Female and male DLCO test results for Control, Obstructive, Restrictive, and Signs & Symptoms group.

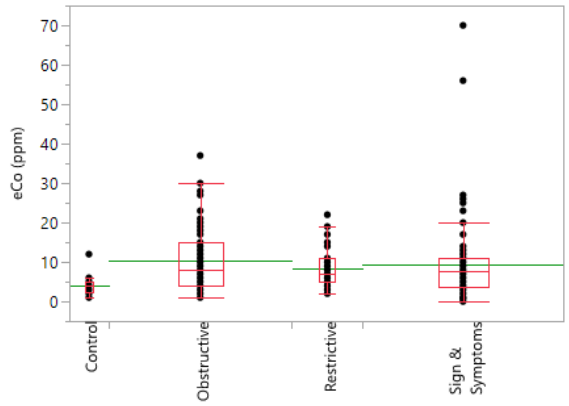
* The green horizontal lines crossing the boxes represent the mean values. The red horizontal lines within the boxes represent the median values. The ends of the boxes represent the 25th and 75th quantiles. The whiskers represent 1.5 x interquartile ranges from the boxes. The disconnected points are potential outliers.

## Levels of Exhaled Carbon Monoxide (eCO)

### *Diagnosis and eCO Levels*

Exhaled CO levels were significantly elevated in both females and males of the obstructive lung disease group, compared to the control group (females: 4.1 ppm, males: 5.0 ppm) and other patients' groups (Figure 10). The mean eCO concentration of the obstructive lung disease group was 10.3 (median: 8.0) ppm (Table 3) in females (n=75, p=0.0002) and 15.3 (median: 9.0) ppm (Table 4) in males (n=62, p=0.0278). The most common diagnosis was asthma (n=61) with a mean eCO of 12.7 (median: 9.0) ppm. The highest levels of eCO were found in patients diagnosed with emphysema with a mean eCO of 17.9 (median: 11.0) ppm (Table 5).

A. Female Group




B. Male group

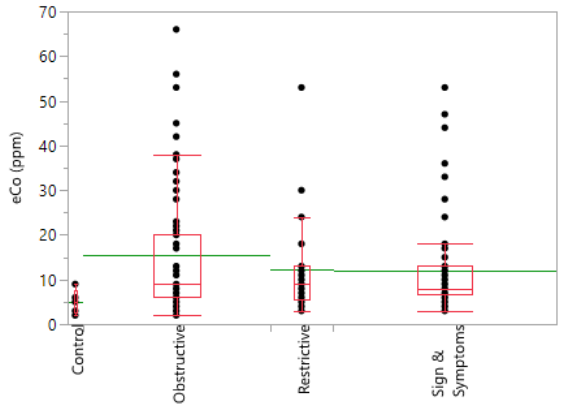



Figure 10. Exhaled carbon monoxide (eCo) results for Control, Obstructive, Restrictive, and Signs & Symptoms group.

* The green horizontal lines crossing the boxes represent the mean values. The red horizontal lines within the boxes represent the median values. The ends of the boxes represent the 25th and 75th quantiles. The whiskers represent 1.5 x interquartile ranges from the boxes. The disconnected points are potential outliers.

Table 5. Distribution of Diagnoses in Obstructive Lung Disease Group (F= Female, M= Male)

| Diagnosis | % of Total | eCO (ppm) | |
|---|---|---|---|
| | | Mean | Median |
| Asthma (n=61; F=33, M=28) | 16.8% (F=9.1%, M=7.7%) | 12.7 (F=11.4, M=14.2) | 9.0 (F=8.0, M=9.0) |
| COPD (n=54; F=28, M=26) | 14.8% (F=7.7%, M=7.1%) | 10.9 (F=10.1, M=11.7) | 8.0 (F=7.5, M=9.0) |
| Bronchitis (n=18; F=8, M=10) | 4.9% (F=2.2%, M=2.7%) | 15.3 (F=8.0, M=21.1) | 8.5 (F=6.5, M=16.5) |
| Emphysema (n=10; F=4, M=6) | 2.7% (F=1.1%, M=1.6%) | 17.9 (F=8.8, M=24.5) | 11.0 (F=7.5, M=20.0) |

Compared with normal subjects, a mild increase was found in levels of eCO among the subjects with restrictive lung disease. The mean eCO concentration was 8.4 (median: 7.0) ppm (Table 3) in females (n=29, p=0.0009) and 12.3 (median: 9.0) ppm (Table 4) in males (n=21, p=0.0397). As shown in Table 6, the most common diagnosis of the restrictive lung disease group was pulmonary fibrosis (n=11) with a mean eCO of 8.7 (median: 7.0) ppm. The highest

levels of eCO were found in patients who had already received or were undergoing chemotherapy (n=7), a mean eCO of 23.7 (median: 15.0) ppm.

Table 6. Distribution of Diagnoses in Restrictive Lung Disease Group (F= Female, M= Male)

| Diagnosis | % of Total | eCO (ppm) | |
|---|---|---|---|
| | | Mean | Median |
| Pulmonary Fibrosis (n=11; F=7, M=4) | 3.2% (F=2.0%, M=1.2%) | 8.7 (F=9.4, M=7.5) | 7.0 (F=10.0, M=7.0) |
| Pulmonary Nodule (n=10; F=3, M=7) | 2.9% (F=0.9%, M=2.0%) | 10.2 (F=5.0, M=12.4) | 6.0 (F=5.0, M=8.0) |
| Chemo Therapy (n=7; F=2 M=5) | 2.1% (F=0.6%, M=1.5%) | 23.7 (F=48.0, M=14.0) | 15.0 (F=48.0, M=15.0) |
| Lung Cancer (n=5; F=3, M=2) | 1.5% (F=0.9%, M=0.6%) | 15.6 (F=7.0, M=28.5) | 8.0 (F=8.0, M=28.5) |

Exhaled CO levels were also mildly elevated in patients admitted with sign and symptoms without a specific diagnosis. The mean eCO concentration was 9.4 (median: 7.5) ppm (Table 3) in females (n=82, p=0.0061) and 12.1 (median: 8.0) ppm (Table 4) in males (n=74 p=0.0201). The most common diagnosis of the signs and symptoms group was shortness of breath (n=68), as shown in Table 7, with a mean eCO of 10.3 (median: 8.0) ppm. The highest levels of eCO were found in patients diagnosed with dyspnea on exertion (n=7), a mean eCO of 14.6 (median: 11.0) ppm

Table 7. Distribution of Diagnoses based in Signs and Symptoms Group (F= Female, M= Male)

| Diagnosis | % of Total | eCO (ppm) | |
|---|---|---|---|
| | | Mean | Median |
| Shortness of Breath (n=68; F=42, M=26) | 18.7% (F=12.2%, M=7.6%) | 10.3 (F=8.4, M=13.3) | 8.0 (F=8.0, M=10.0) |
| Dyspnea (n=36; F=22, M=14) | 10.5% (F=6.4%, M=4.1%) | 9.9 (F=8.0, M=12.9) | 8.5 (F=7.0, M=10.0) |
| Chronic Cough (n=35; F=19, M=16) | 10.2% (F=5.5%, M=4.7%) | 10.0 (F=8.4, M=11.9) | 8.0 (F=7.0, M=9.0) |
| Dyspnea on Exertion (n=26; F=17, M=9) | 7.6% (F=5.0%, M=2.6%) | 14.6 (F=13.7, M=16.3) | 11.0 (F=11.0, M=11.0) |

***Smoking History and eCO***

Smoking causes an acute increase in exhaled breath CO, making this measurement less useful in this group of patients, and as expected, smoking subjects had a higher CO concentration in exhaled air than nonsmoking subjects. The eCO levels of nonsmokers, smokers, and ex-smokers in each disease group are shown in Table 8. The mean eCO concentration of the control group was 3.5 (median: 4.0) ppm in nonsmoking female subjects (n=15) and 4.8 (median: 4.0) ppm in nonsmoking male subjects (n=4).

Exhaled CO levels were markedly elevated in current smokers with obstructive lung disease. The mean eCO was 13.5 (median: 14.5) ppm in females (n=10) and 27.5 (median: 18.0) ppm in males (n=13). Exhaled CO concentration in nonsmoking subjects was mildly increased. The mean eCO in females (n=63) was 10.0 (median: 7.0) ppm and 12.1 (median: 8.0) in males of nonsmoking subjects (n=49). Exhaled CO concentration in subjects who quit smoking, ex-smokers, was also increased. The mean eCO was 10.0 (median: 7.5) ppm in females (n=28) and 14.0 (median: 9.0) ppm in males (n=33). As shown in Figure 11 and 12, nonsmokers and ex-smokers, both females and males, in the obstructive lung disease group produced higher eCO levels than the other two groups.

The results of exhaled CO levels of the restrictive lung disease group were not statistically significant since only one female and 5 males from the group were current smokers. The mean eCO of the smoking female subject was 6.0 ppm, and the mean eCO concentration of the smoking male subjects was 23.8 (median: 18.0) ppm. Exhaled CO concentration in nonsmoking subjects was slightly increased. The mean eCO in nonsmoking female subjects (n=28) was 8.5 (median: 7.5) ppm and 8.8 (median: 7.5) ppm in male subjects (n=16). Exhaled CO concentration in former smokers was not significantly increased. The mean eCO in female

ex-smokers (n=10) was 9.1 (median: 5.5) ppm and 7.4 (median: 7.0) ppm in male ex-smokers (n=11).

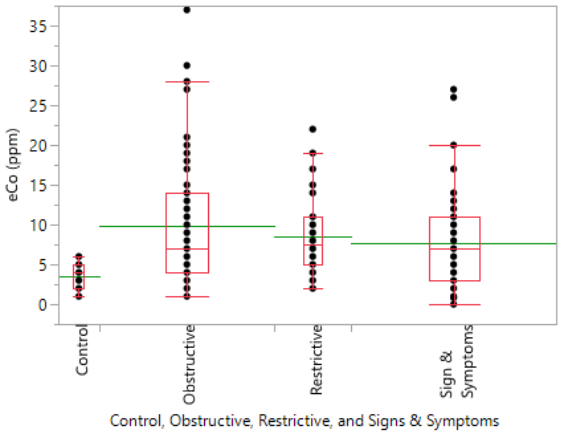
Exhaled CO levels were markedly elevated in the current smokers of the Signs and Symptoms group. The mean eCO concentration was 37.4 (median: 25.0) ppm in female subjects (n=5) and 23.8 (median: 24.0) ppm in male subjects (n=9) of the group. Exhaled CO concentration in nonsmoking subjects was mildly increased. The mean eCO in females (n=75) was 7.6 (median: 7.0) ppm and 10.5 (median: 8.0) ppm in males (n=65). Exhaled CO concentration in ex-smokers in this group was also slightly increased. The mean eCO concentration was 8.0 (median: 8.0) ppm in females (n=20) and 9.5 (median: 7.5) ppm in males (n=28).

Table 8. Smoking History & eCO Levels (ppm) of Female Participants

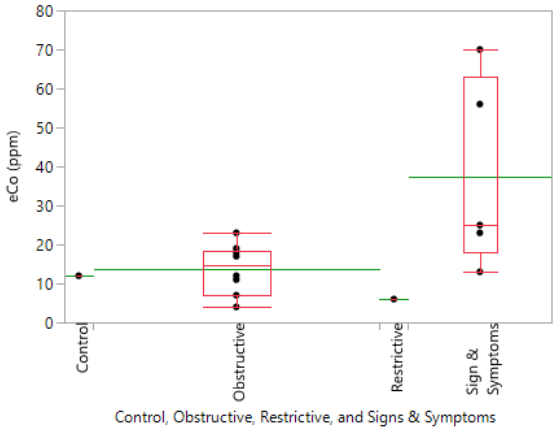
| **Participants** | **Current Smokers** | **Non-Smokers** | **Former Smokers** | **Unknown** |
|---|---|---|---|---|
| **Control Group** (N=16) | 12.0 (n=1,eCig) | 3.5±0.4 (n=15) | 3.5±1.5 (n=2) | |
| **Obstructive Lung Disease Group** (N=75) | 13.5±2.0[1] (n=10) | 9.9±1.0 (n=64)[c] | 10.0±1.4 (n=28) | (n=1) |
| **Restrictive Lung Disease Group** (N=29) | 6.0 (n=1) | 8.5±1.0 (n=28)[c] | 9.1±2.0 (n=10) | |
| **Signs & Symptoms Group** (N=82) | 37.4±10.9 (n=5) | 7.6±0.6 (n=75)[c] | 8.0±1.1 (n=20) | (n=2) |

[1]Expressed as mean ± SEM of eCO levels. Superscripts on PFT results indicate levels of statistical significance for comparisons of values within rows: [a]P < 0.05; [b]P < 0.01; [c]P < 0.005; [d]P < 0.0001; no superscript, P > 0.05.

A. Female nonsmokers




B. Female smokers




C. Female former smokers

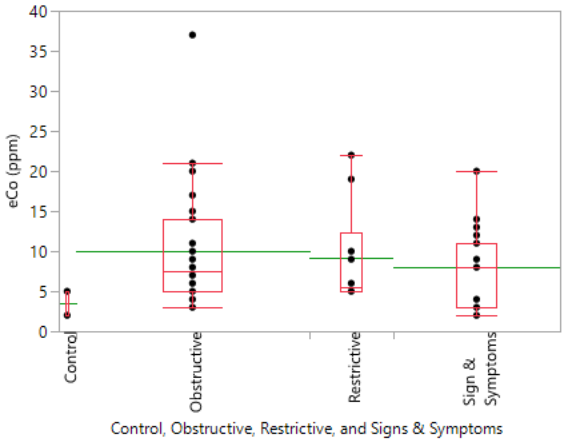


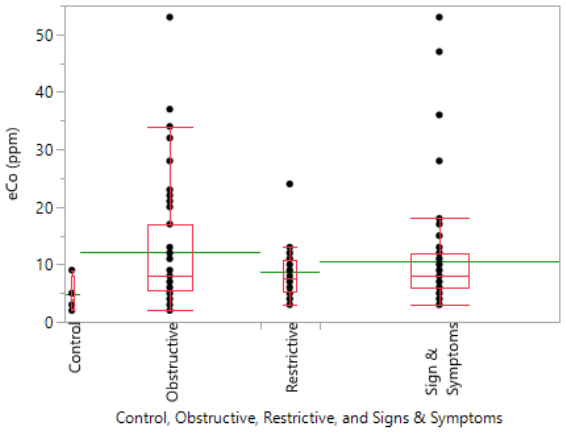

Figure 11. Exhaled carbon monoxide (eCo) results for ***female*** nonsmokers (A), smokers (B), and ex-smokers (C).

Table 9. Smoking History & eCO Levels (ppm) of Male Participants

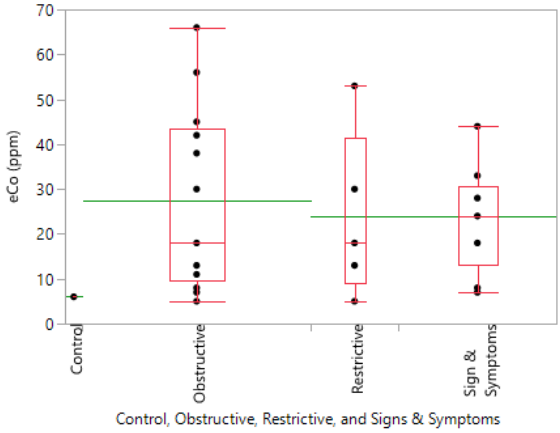
| **Participants** | **Current Smokers** | **Non-Smokers** | |
|---|---|---|---|
| | | | **Former Smokers** |
| **Control Group** (N=5) | 6.0 (n=1, eCig) | 4.8±1.5 (n=4) | 2.5±0.5 (n=2) |
| **Obstructive Lung Disease Group** (N=62) | 27.5±5.6[1] (n=13) | 12.1±1.5 (n=49) | 14.0±2.3 (n=33) |
| **Restrictive Lung Disease Group** (N=21) | 23.8±8.4 (n=5) | 8.8±1.2 (n=16) | 7.4±0.9 (n=11) |
| **Signs & Symptoms Group** (N=74) | 23.8±3.9 (n=9) | 10.5±1.1 (n=65) [c] | 9.5±1.7 (n=28) |

[1]Expressed as mean ± SEM of eCO levels. Superscripts on PFT results indicate levels of statistical significance for comparisons of values within rows: [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.005$; [d]$P < 0.0001$; no superscript, $P > 0.05$.

A. Male nonsmokers




B. Male smokers




C. Male ex-smokers

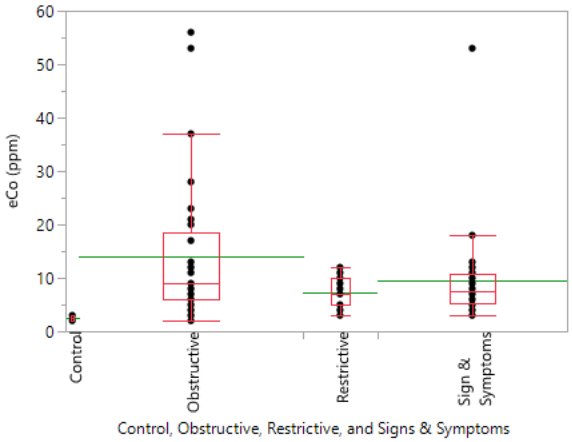



Figure 12. Exhaled carbon monoxide (eCo) results for ***male*** nonsmokers (A), smokers (B), and ex-smokers (C).

### *Use of Anti-inflammatory Drugs and eCO*

Compared with the control group, the levels of exhaled CO were increased in the subjects with the obstructive lung disease, restrictive lung disease, and signs and symptoms, both in those treated with anti-inflammatory drugs and those not treated with anti-inflammatory drugs (Table 10 and 11). This result suggests that increased eCO level may be an indicator of lung

inflammation, but the anti-inflammatory drugs were not effective in the down-regulation of heme oxygenase-1 (HO-1).

The exhaled CO concentration was significantly higher in the subjects with obstructive lung disease receiving anti-inflammatory drugs. The mean eCO concentration was 12.3 (median: 9.0) ppm (Table 10) in females (n=33) and 13.3 (median: 9.0) ppm (Table 11) in males (n=25). The mean eCO level of the subjects who were not receiving anti-inflammatory drugs was 8.8 (median: 6.0) ppm (Table 10) in females (n=42) and 16.7 (median: 8.0) ppm (Table 11) in males (n=37).

In subjects with restrictive lung disease receiving anti-inflammatory drugs, the exhaled CO was not significantly increased compared to the control group. The mean eCO concentration of the subjects receiving anti-inflammatory drugs was 7.4 (median: 5.0) ppm (Table 10) in females (n=7) and 25.0 (median: 13.0) ppm (Table 11) in males (n=3). The mean eCO level of the subjects who were not receiving anti-inflammatory drugs was 8.8 (median: 8.5) ppm (Table 10) in females (n=22) and 10.2 (median: 7.5) ppm (Table 11) in males (n=18).

In subjects diagnosed with signs and symptoms, female subjects did not differ significantly from that in the control group while male subjects showed markedly increased eCO levels. The mean eCO concentration of the female subjects who were receiving anti-inflammatory drugs (n=13) was 6.4 (median: 6.0) ppm (Table 10). Exhaled CO concentration in female subjects of the group not receiving anti-inflammatory drugs (n=69) was mildly increased with a mean eCO of 9.9 (median: 8.0) ppm (Table 10). Male subjects who were receiving anti-inflammatory drugs (n=6) had higher exhaled CO concentration with a mean eCO of 18.3 (median: 12.0) ppm than males not receiving anti-inflammatory drugs (n=68) with a mean eCO of 11.5 (median: 8.0) ppm (Table 11).

Table 10. eCO Levels (ppm) of female participants as related to the use of anti-inflammatory drugs

| | **Use of Anti-Inflammatory Drugs** | |
|---|---|---|
| | **Yes** | **No** |
| Control Group (N=16) | N/A | 4.1±0.6 (n=16) |
| Obstructive Lung Disease Group (N=75) | 12.3±1.8 (n=33) | 8.8±1.0 [c] (n=42) |
| Restrictive Lung Disease Group (N=29) | 7.4±2.1 (n=7) | 8.8±1.1[c] (n=22) |
| Signs & Symptoms **Group** (N=82) | 6.4±1.2 [a] (n=13) | 9.9±1.3 [b] (n=69) |

[1]Expressed as mean ± SEM of eCO levels. Superscripts on PFT results indicate levels of statistical significance for comparisons made of values within rows: [a]P < 0.05; [b]P < 0.01; [c]P < 0.005; [d]P < 0.0001; no superscript, P > 0.05.

Table 11. eCO Levels (ppm) of male participants as related to the use of anti-inflammatory drugs

| | **Use of Anti-Inflammatory Drugs** | |
|---|---|---|
| | **Yes** | **No** |
| **Control Group** (N=16) | N/A | 5.0±1.2 (n=5) |
| **Obstructive Lung Disease Group** (N=75) | 13.3±2.3 (n=25) | 16.7±2.7 [a] (n=37) |
| **Restrictive Lung Disease Group** (N=29) | 25.0±14.0 (n=3) | 10.2±1.7 (n=18) |
| **Signs & Symptoms Group** (N=82) | 18.3±7.1 (n=6) | 11.5±1.1 [a] (n=68) |

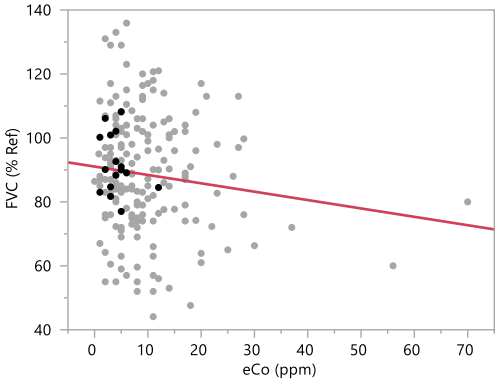
[1]Expressed as mean ± SEM of eCO levels. Superscripts on PFT results indicate levels of statistical significance for comparisons of values within rows: [a]P < 0.05; [b]P < 0.01; [c]P < 0.005; [d]P < 0.0001; no superscript, P > 0.05.

## Correlations between PFT and eCO

### Relations between Spirometry and eCO

There was a tendency for a negative correlation between spirometry measurements and eCO results. FVC (% Ref) decreased with elevated concentrations of eCO levels in females (p=0.0410); however, there was no significant correlation in males (p=0.5755) as shown in Figure 13 and 14. The females FEV1 (% Ref) also decreased with elevated eCO levels in females (p=0.0119) and mildly decreased in males (p=0.2135) (Fig. 15 and 16). FEV1/FVC (%) in both females (p=0.0109) and males (p=0.0284) also had a significant negative correlation with eCO results (Fig. 17 and 18), suggesting eCO levels increases with the obstructive disease in progress. $FEF_{25-75\%}$, representing small airway, had the greatest negative correlation with eCO levels in both females (p=0.0089) and males (p=0.0173), as shown Figure 19 and 20, suggesting small airways are most susceptible areas for inflammation for both females and males. It is worth to mention that there was a more significant negative correlation between the spirometry values and eCO levels in females than males as shown in Figure 14, 16, 18, and 20.

A. Female Group




B. Male Group

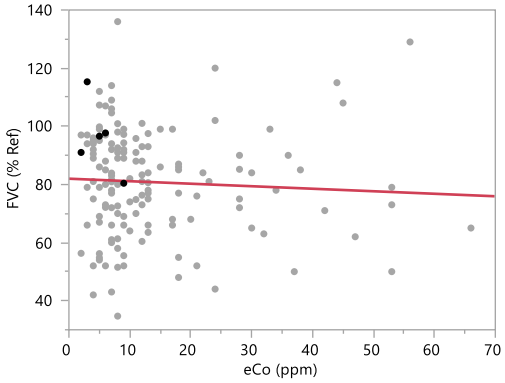



Figure 13. Relation between exhaled CO and FVC (% Ref) in females (r=-0.13; p=0.0410) (panel A) and males (r=-0.04; p=0.5775) (panel B). (Darker markers = Control group).

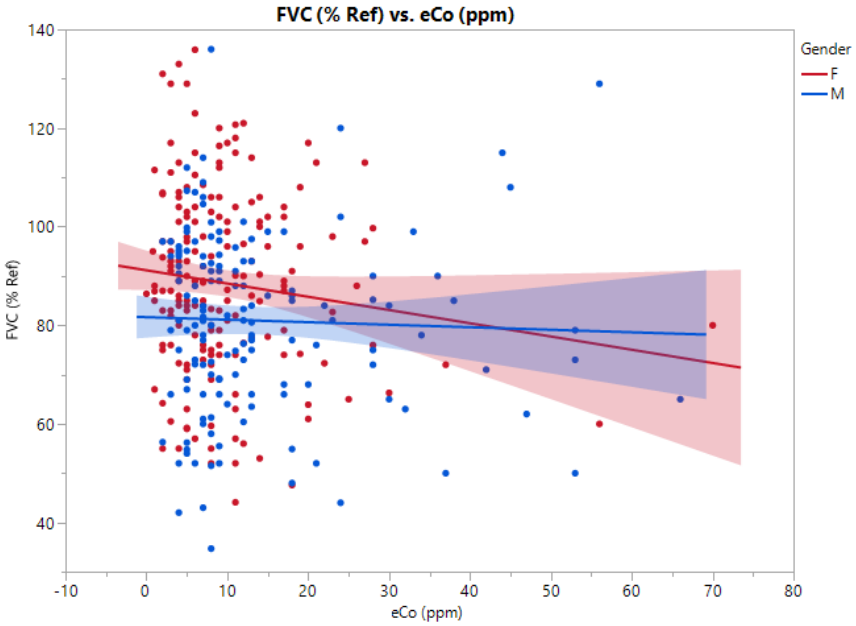



Figure 14. Relation between exhaled CO and FVC(% Ref) in both females and males.

A. Female Group

B. Male Group

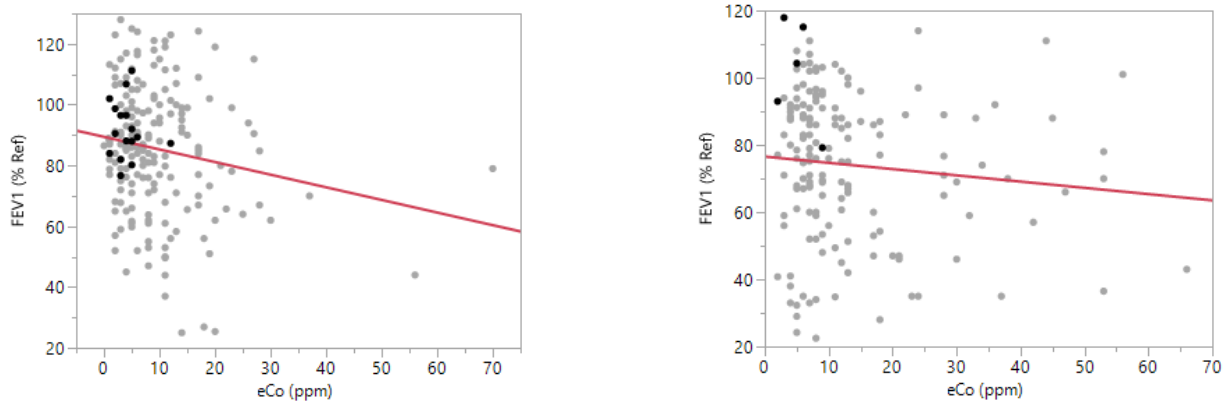



Figure 15. Relation between exhaled CO and FEV1 (% Ref) in females (r=-0.18; p=0.0119) (panel A) and males (r=-0.10; p=0.2135) (panel B). (Darker markers = Control group).

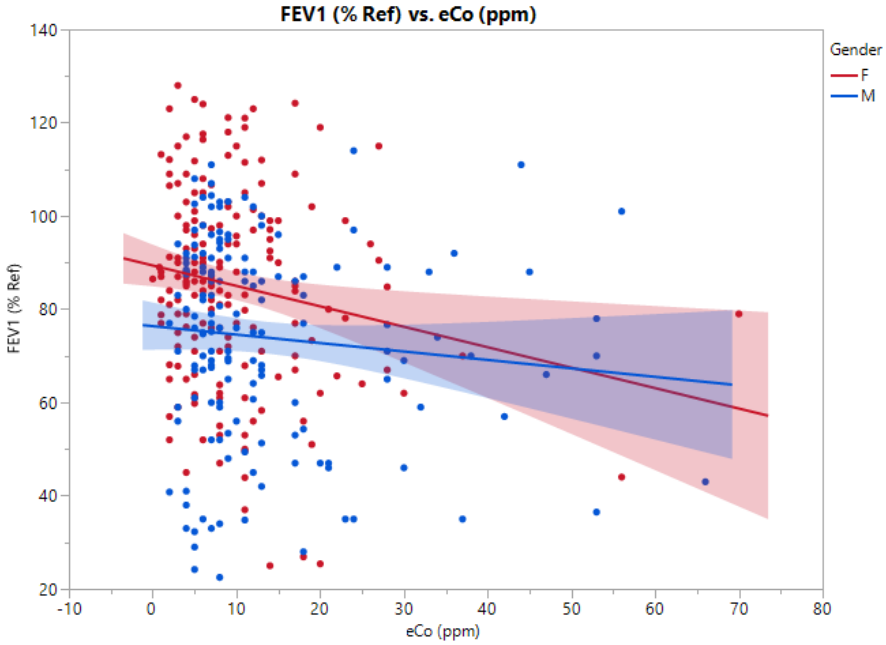



Figure 16. Relation between exhaled CO and FEV1(% Ref) in both females and males.

A. Female Group

B. Male Group

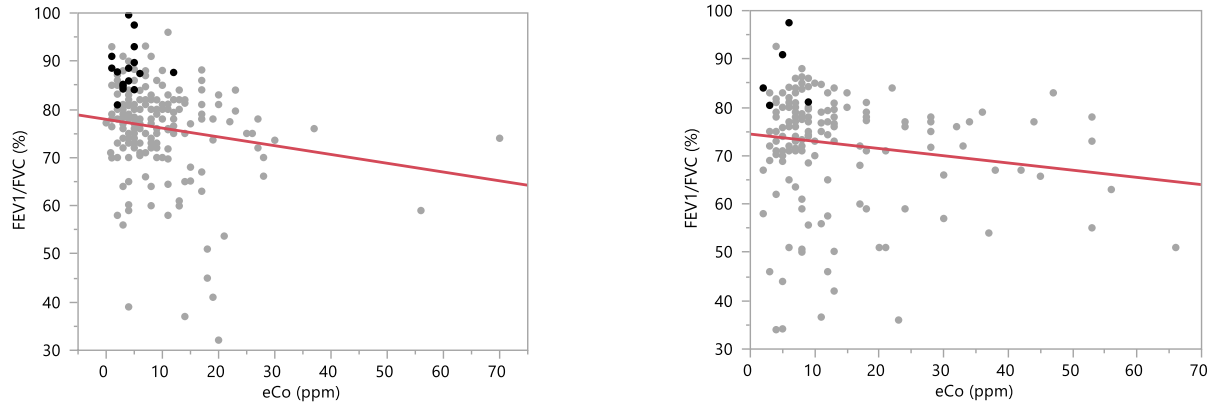



Figure 17. Relation between exhaled CO and FEV1/FVC (%) in females (r=-0.20; p=0.0109) (panel A) and males (r=-0.13; p=0.0284) (panel B). (Darker markers = Control group)

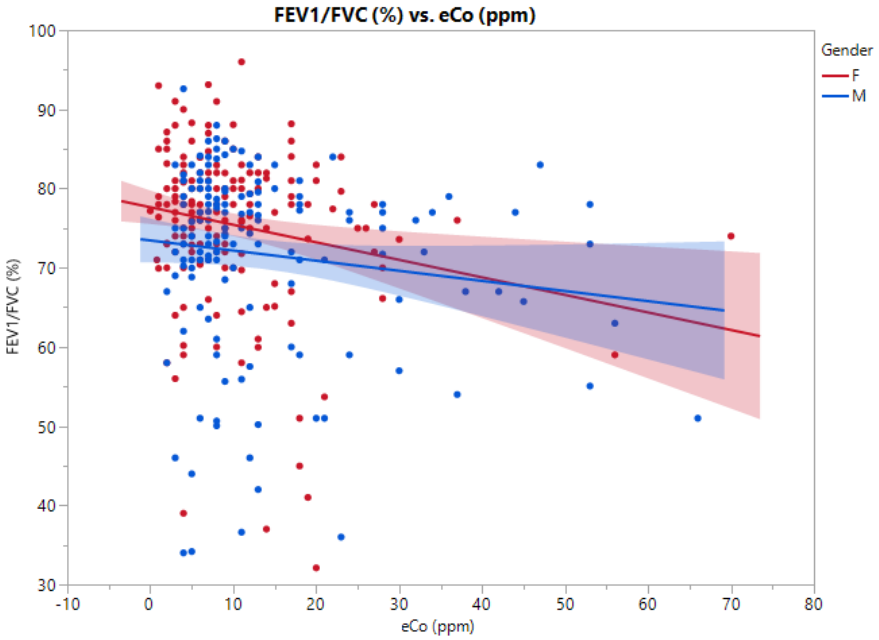
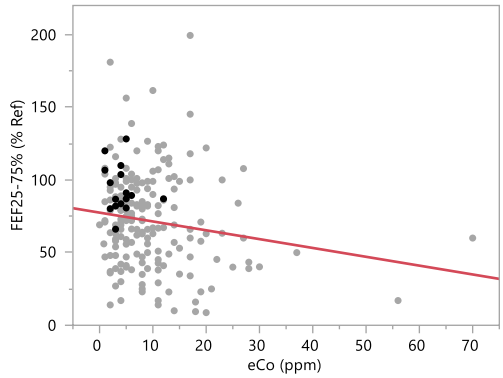



Figure 18. Relation between exhaled CO and FEV1/FVC (%) in both females and males.

A. Female Group




B. Male Group

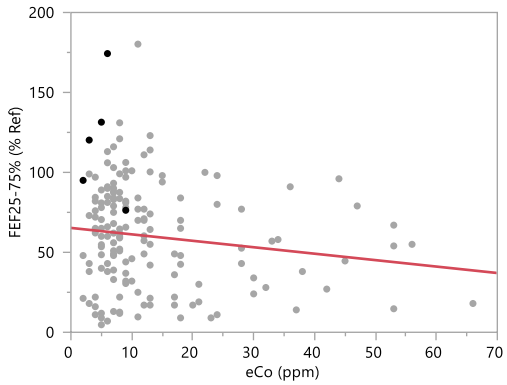



Figure 19. Relation between exhaled CO and $FEF_{25-75\%}$ (% Ref) in females (r=-0.16; p=0.0089) (panel A) and males (r=-0.16; p=0.0173) (panel B). (Darker markers = Control group)

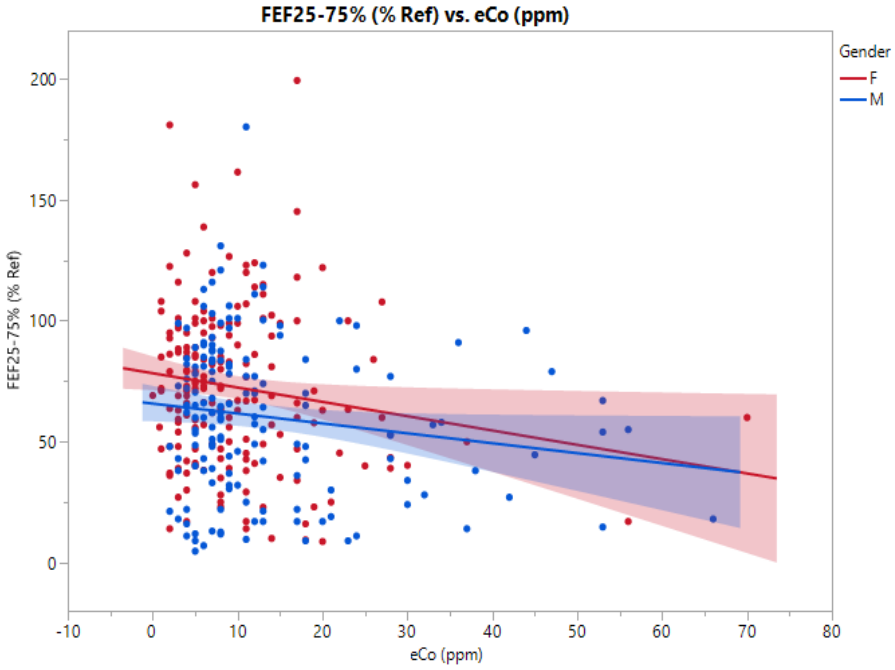
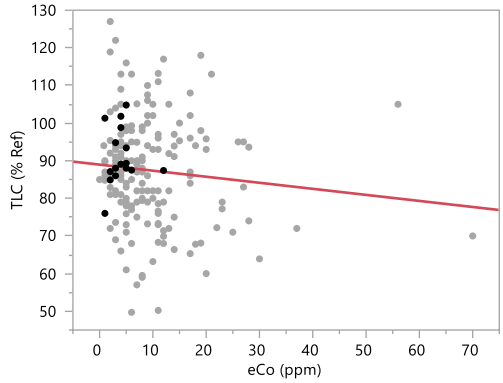



Figure 20. Relation between exhaled CO and $FEF_{25-75\%}$ (%Ref) in both females and males.

**Relations between lung volume test and eCO**

As Figures 21-24 show, lung volume test results showed the opposite correlation between females and males. The female group had a negative correlation between lung volume test and eCO levels, as shown in Fig. 21 and 23, while the male group had a positive correlation. With increased levels of eCO, total lung capacity (TLC) and functional residual capacity (FRC) of the female group decreased while those of the male group increased.

A. Female Group




B. Male Group

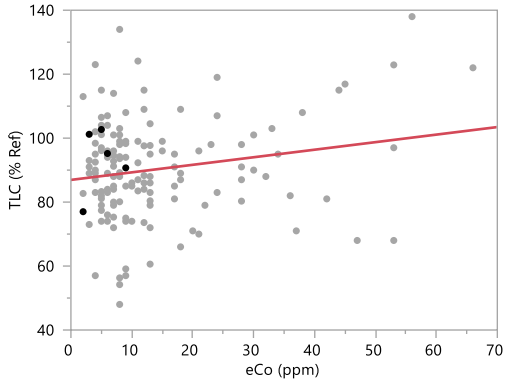



Figure 21. Relation between exhaled CO and TLC (% Ref) in females (r=-0.10; p=0.1998) (panel A) and males (r=0.20; p=0.1270) (panel B). (Darker markers = Control group)

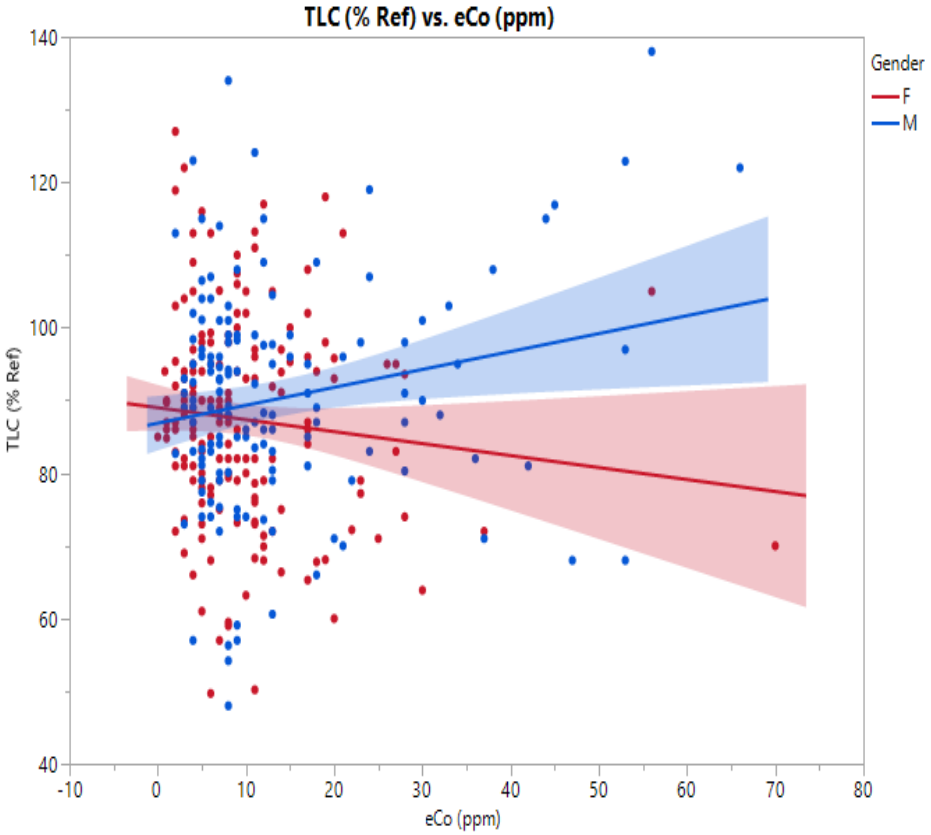
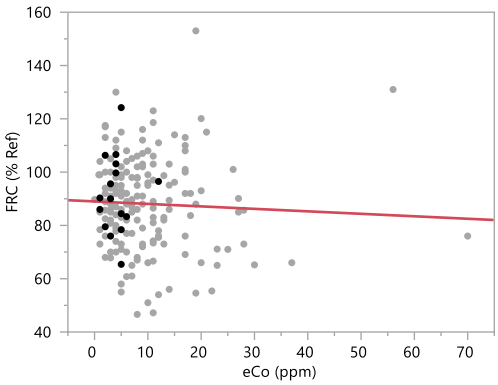



Figure 22. Relation between exhaled CO and TLC (% Ref) in both females and males.

A. Female Group




B. Male Group

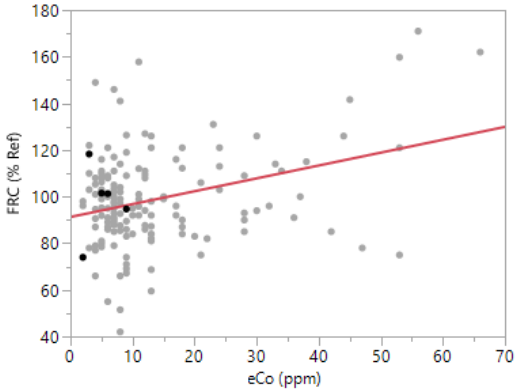



Figure 23. Relation between exhaled CO and FRC (% Ref) in females (r=-0.02; p=0.6341) (panel A) and males (r=0.33; p=0.0233) (panel B). (Darker markers = Control group)

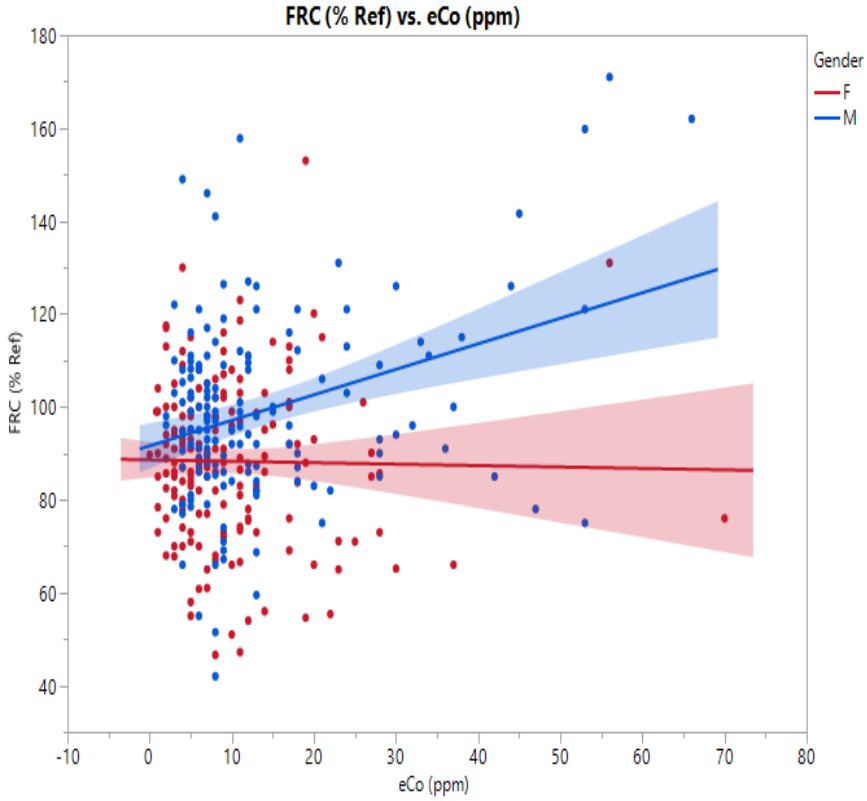
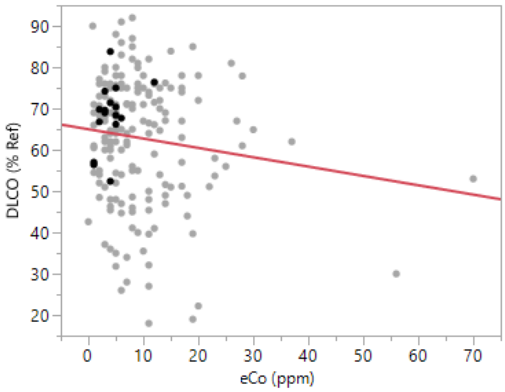



Figure 24. Relation between exhaled CO and FRC (% Ref) in both females and males

**Relations between DLCO and eCO**

Diffusing capacity of lungs for carbon monoxide (DLCO) test results also showed opposite correlations with eCO between females and males, with a negative correlation for the female group and a slight positive correlation for the male group (Fig. 25 and 26).

A. Female Group




B. Male Group

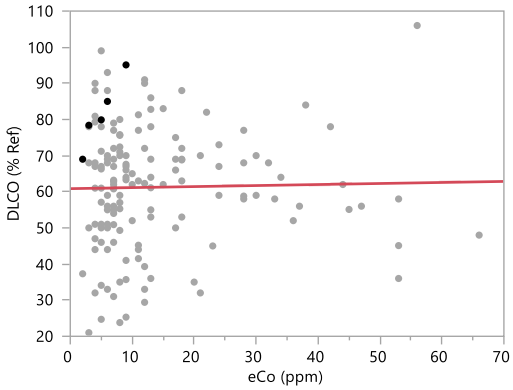



Figure 25. Relation between exhaled CO and DLCO (Ref %) in females (r=-0.14; p=0.0532) (panel A) and males (r=0.04; p=0.8216) (panel B). (Darker markers = Control group)

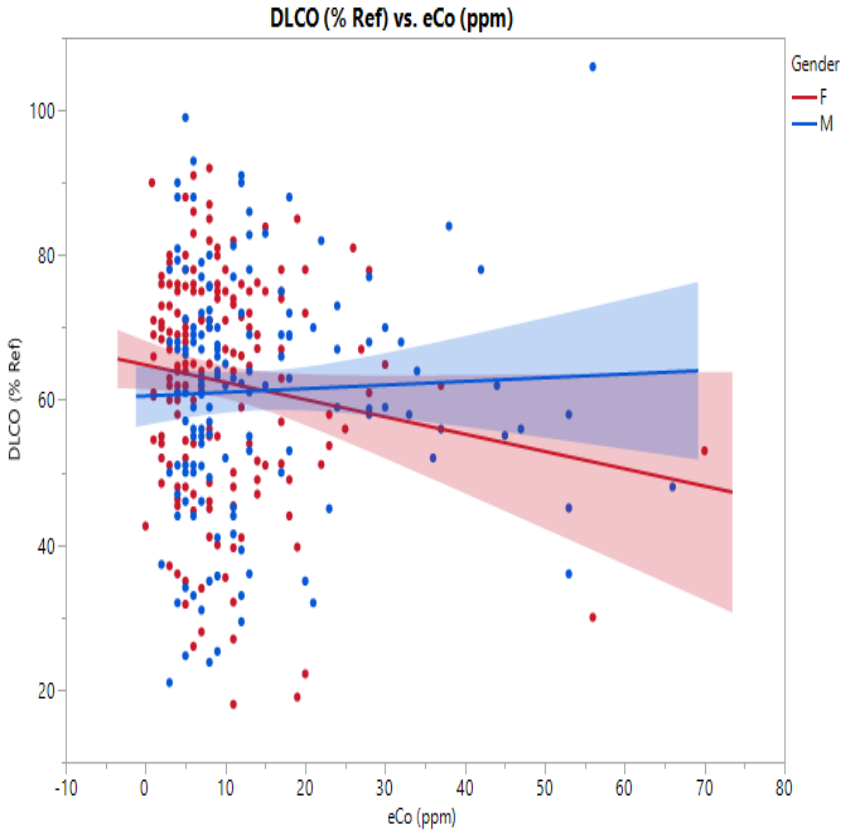
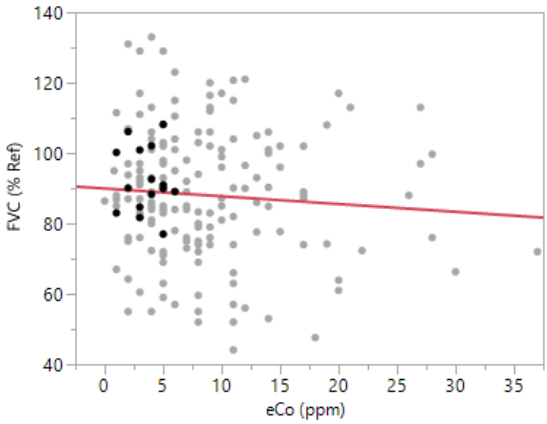



Figure 26. Relation between exhaled CO and DLCO (% Ref) in both females and males.

**Relations between Spirometry and eCO by Smoking Status**

For female smokers, there were significant negative correlations between spirometry measures and eCO. These associations were not as consistent in male smokers. FVC (% Ref) decreased with elevated concentrations of eCO levels in female smokers (r=-0.54; p=0.0108) (Fig. 27) while there was an insignificant positive correlation for male smokers (r=0.15; p=0.4645) (Fig. 28). There was a strong negative correlation between FVC and eCO (Fig. 29).

A. Female nonsmokers Group




B. Female smokers Group

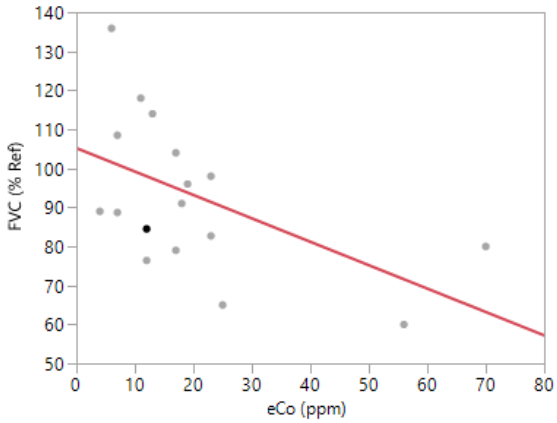
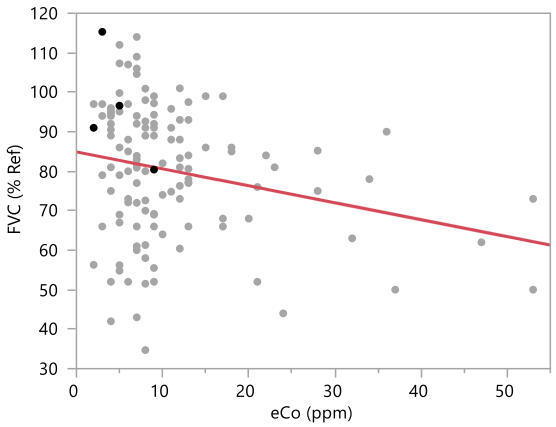



Figure 27. Relation between exhaled CO and FVC (% Ref) in ***female nonsmokers*** (r=-0.08; p=0.2930) (panel A) and ***female smokers*** (r=-0.54; p=0.0108) (panel B). (Darker markers = Control group)

A. Male nonsmokers Group




B. Male smokers Group

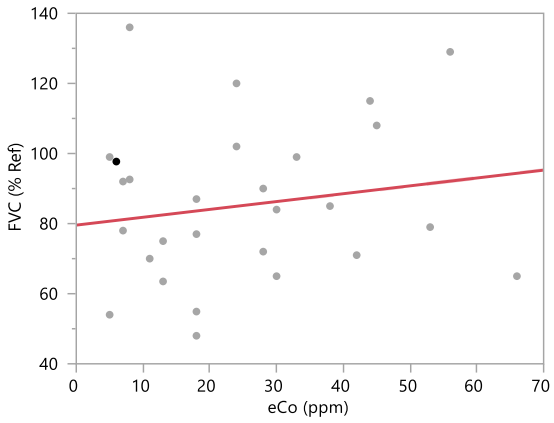



Figure 28. Relation between exhaled CO and FVC (% Ref) in ***male nonsmokers*** (r=-0.24; p=0.0009) (panel A) and ***male smokers*** (r=0.15; p=0.4645) (panel B). (Darker markers = Control group)

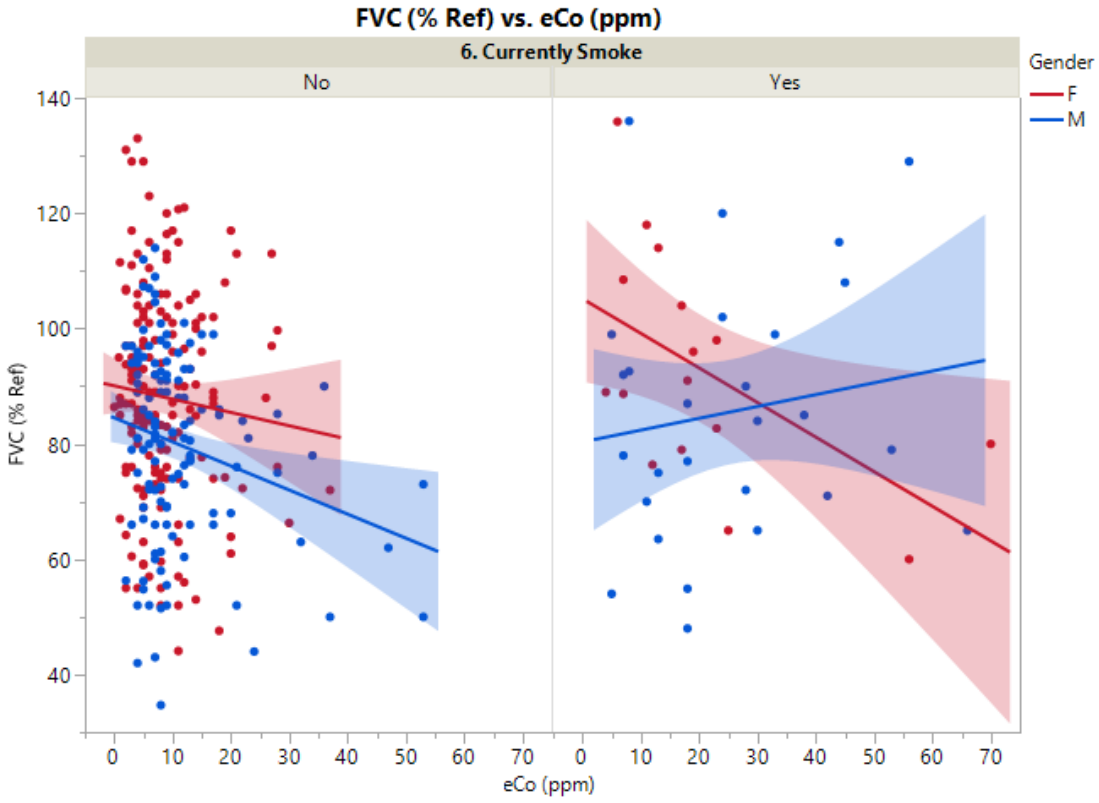
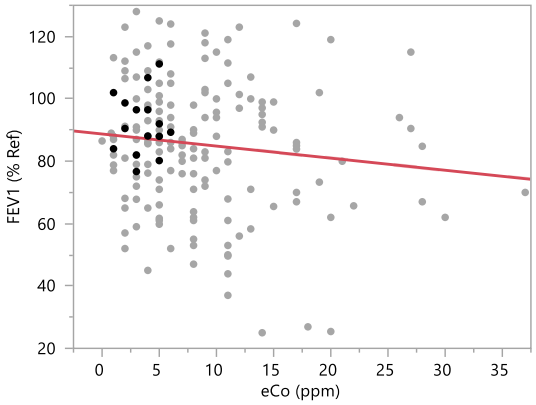



Figure 29. Relation between exhaled CO and FVC (% Ref) in nonsmokers and smokers.

FEV1 (% Ref) decreased with elevated eCO levels in female smokers (r=-0.48; p=0.0392) (Fig. 30), and it showed little association with eCO in male smokers (p=0.7271) (Fig. 31), yielding a similar pattern as the relationship between FVC (% Ref) and eCO levels (Fig. 32).

A. Female nonsmokers Group




B. Female smokers Group

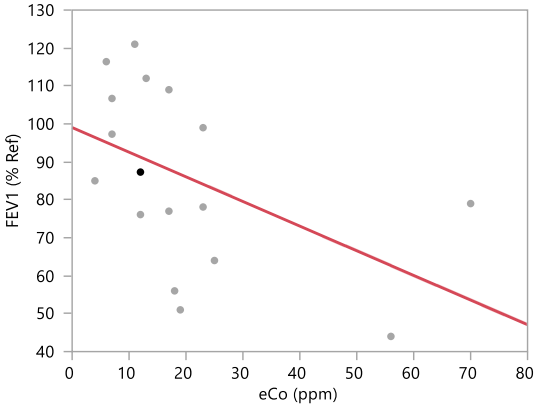
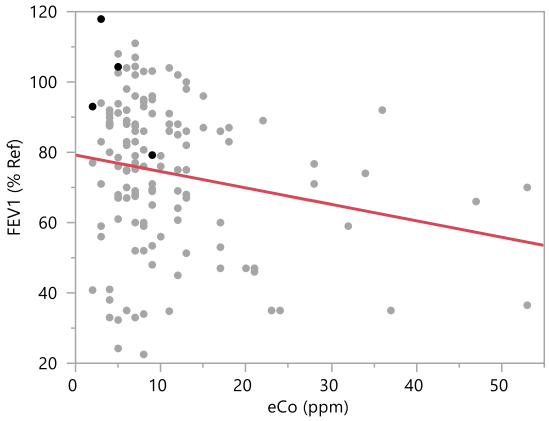



Figure 30. Relation between exhaled CO and FEV1 (% Ref) in ***female nonsmokers*** (r=--0.14; p=0.1038) (panel A) and ***female smokers*** (r=-0.48; p=0.0392) (panel B). (Darker markers = Control group)

A. Male nonsmokers Group




B. Male smokers Group

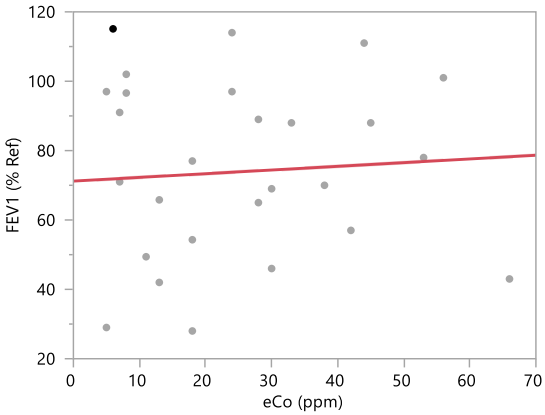



Figure 31. Relation between exhaled CO and FEV1 (% Ref) in ***male nonsmokers*** (r=-0.20; p=0.0114) (panel A) and ***male smokers*** (r=0.07; p=0.7271) (panel B). (Darker markers = Control group)

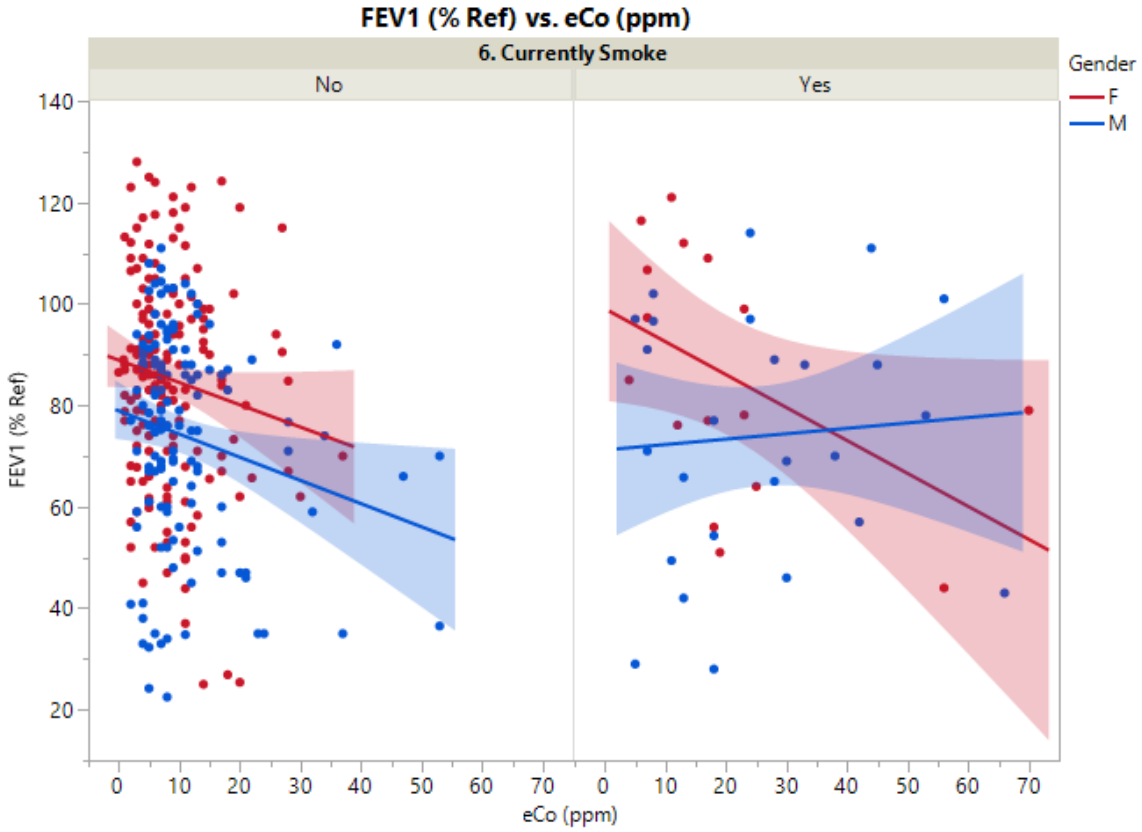
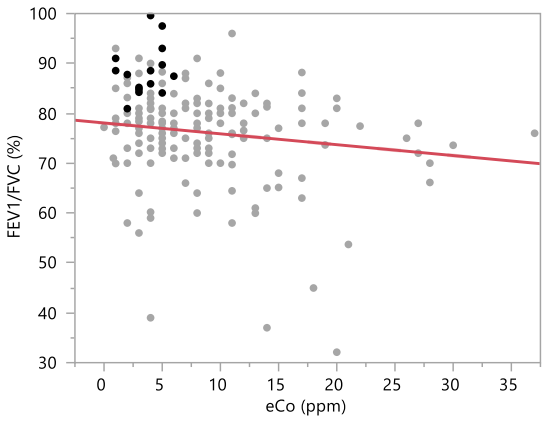



Figure 32. Relation between exhaled CO and FEV1 (% Ref) in nonsmokers and smokers.

FEV1/FVC (%) in both females and males had negative correlations with eCO. It moderately decreased for female smokers (p=0.1056) (Fig. 33) and mildly decreased for male smokers (p=0.6316) (Fig. 34) with elevated eCO levels. There were stronger negative correlations for females, as shown in Fig. 35, between FEV1/FVC (%) and eCO test values.

A. Female nonsmokers Group




B. Female smokers Group

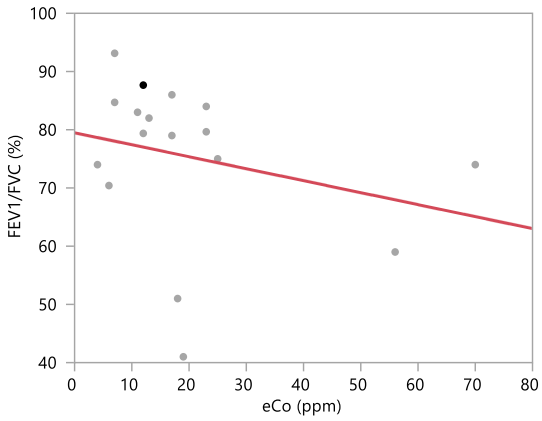
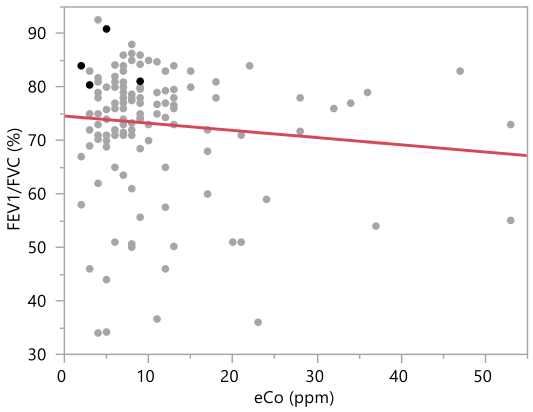



Figure 33. Relation between exhaled CO and FEV1/FVC (%) in ***female nonsmokers*** (r=-0.19; p=0.0198) (panel A) and ***female smokers*** (r=-0.27; p=0.1056). (Darker markers = Control group)

A. Male nonsmokers Group




B. Male smokers Group

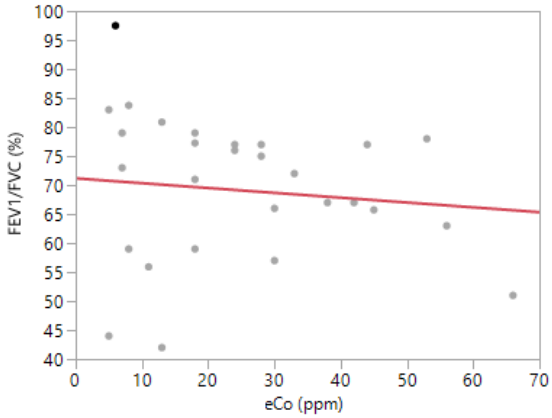



Figure 34. Relation between exhaled CO and FEV1/FVC (%) in ***male nonsmokers*** (r=-0.09; p=0.2159) (panel A) and ***male smokers*** (r=-0.10; p=0.6316) (panel B). (Darker markers = Control group)

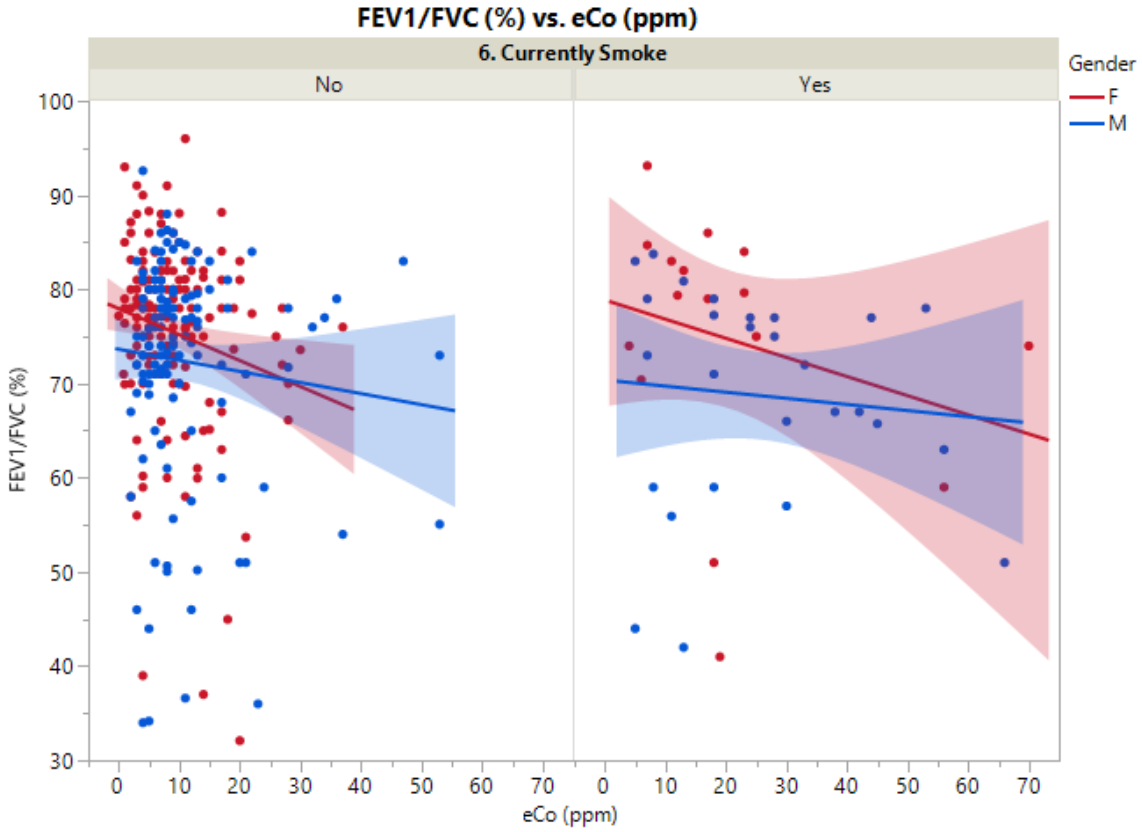
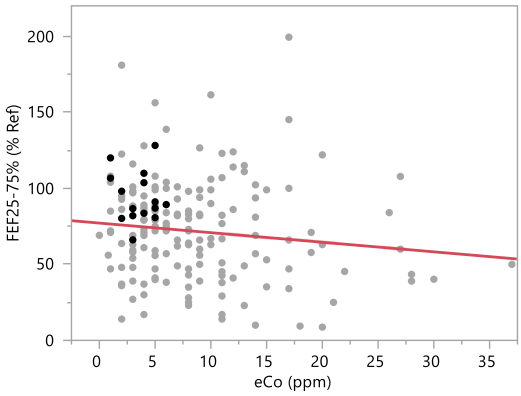



Figure 35. Relation between exhaled CO and FEV1/FVC (% Ref) in nonsmokers and smokers.

$FEF_{25-75\%}$ also markedly decreased in female smokers (p=0.0374) (Fig. 36); however, there was no significantly different relationship between eCO levels and $FEF_{25-75\%}$ among male smokers (p=0.5959) (Fig. 37 and 38).

A. Female nonsmokers Group




B. Female smokers Group

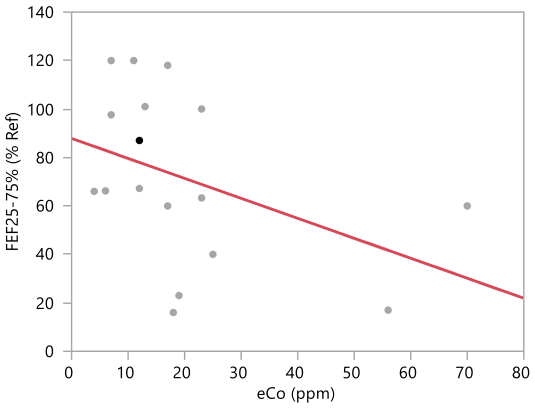
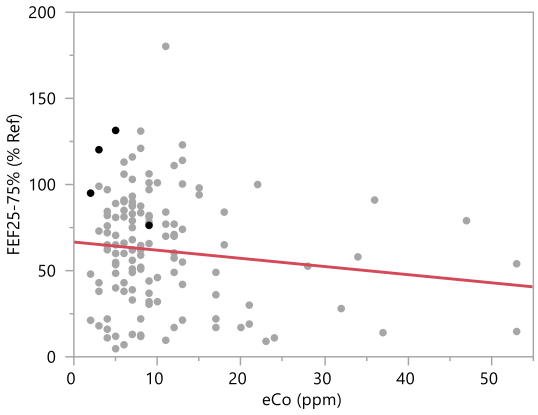



Figure 36. Relation between exhaled CO and $FEF_{25-75\%}$ (% Ref) in ***female nonsmokers*** (r=-0.11; p=0.0707) (panel A) and ***female smokers*** (r=-0.42; p=0.0374) (panel B). (Darker markers = Control group)

A. Male nonsmokers Group




B. Male smokers Group

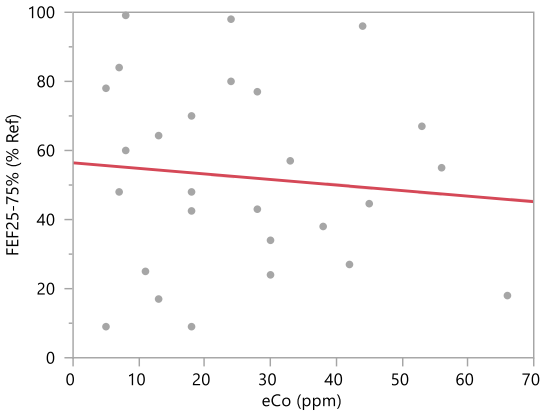



Figure 37. Relation between exhaled CO and $FEF_{25-75\%}$ (% Ref) in ***male nonsmokers*** (r=-0.14; p=0.0795) (panel A) and ***male smokers*** (r=-0.10; p=0.5959) (panel B). (Darker markers = Control group)

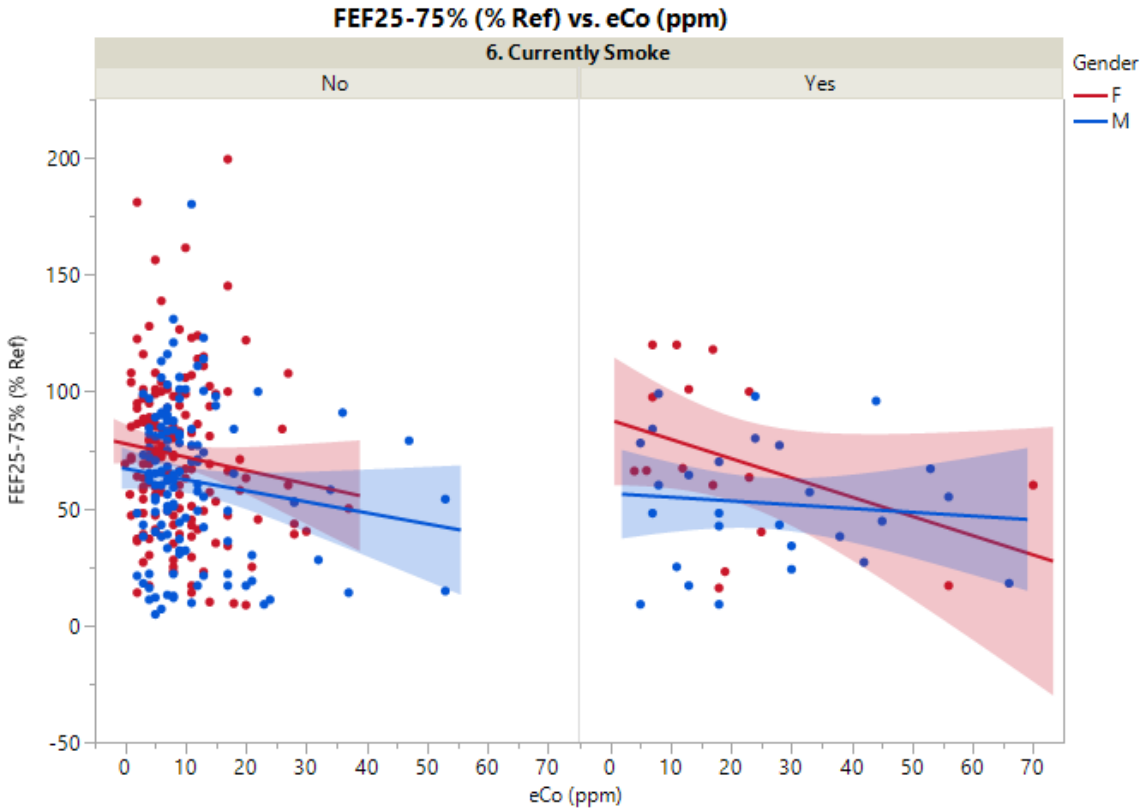
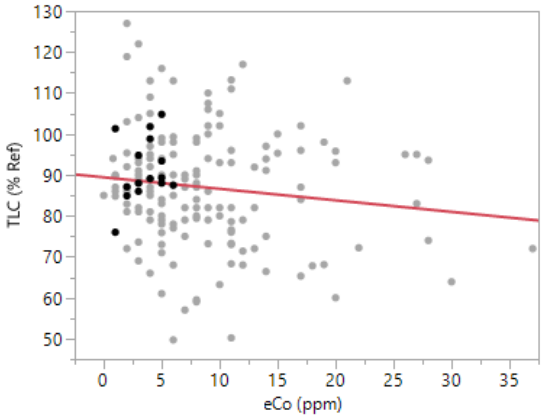



Figure 38. Relation between exhaled CO and $FEF_{25-75\%}$ (% Ref) in nonsmokers and smokers.

Lung volume test (% Ref) for smokers also showed the opposite correlation between females and males. There was no significant different relationship between eCO levels and TLC (% Ref) among female smokers (p=0.2859) (Fig. 39) although correlations were negative for the female group. There was a significant positive correlation among male smokers (p=0.0204) between eCO levels and TLC (% Ref) (Fig. 40). Overall, there were opposite correlations for females and males, negative correlations for females and positive correlations for males (Fig. 41).

A. Female nonsmokers Group




B. Female smokers Group

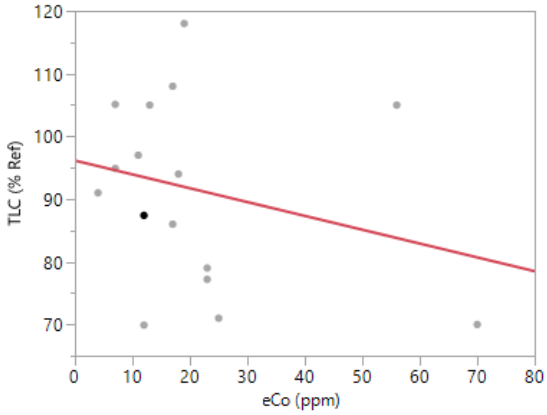
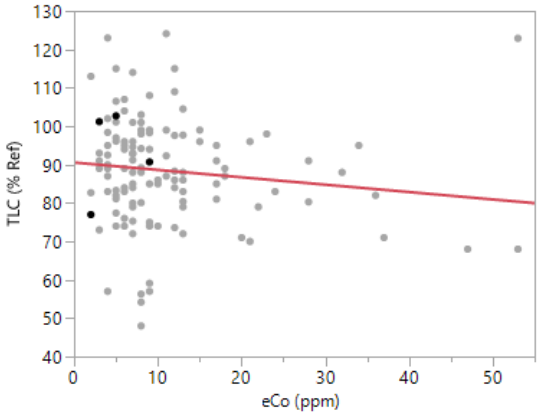



Figure 39. Relation between exhaled CO and TLC (% Ref) in ***female nonsmokers*** (r=-0.14; p=0.0777) (panel A) and ***female smokers*** (r=-0.26; p=0.2859) (panel B). (Darker markers = Control group)

A. Male nonsmokers Group




B. Male smokers Group

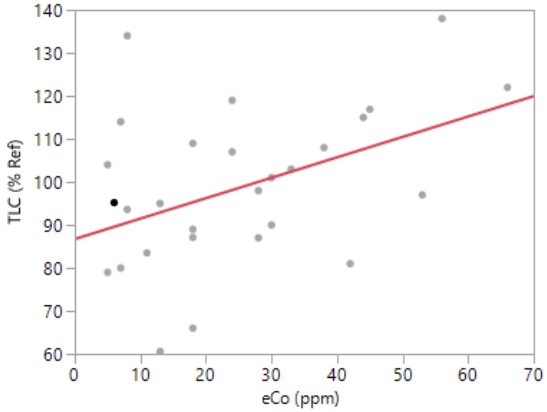



Figure 40. Relation between exhaled CO and TLC (% Ref) in ***male nonsmokers*** (r=-0.08; p=0.2898) (panel A) and ***male smokers*** (r=0.41; p=0.0204) (panel B). (Darker markers = Control group)

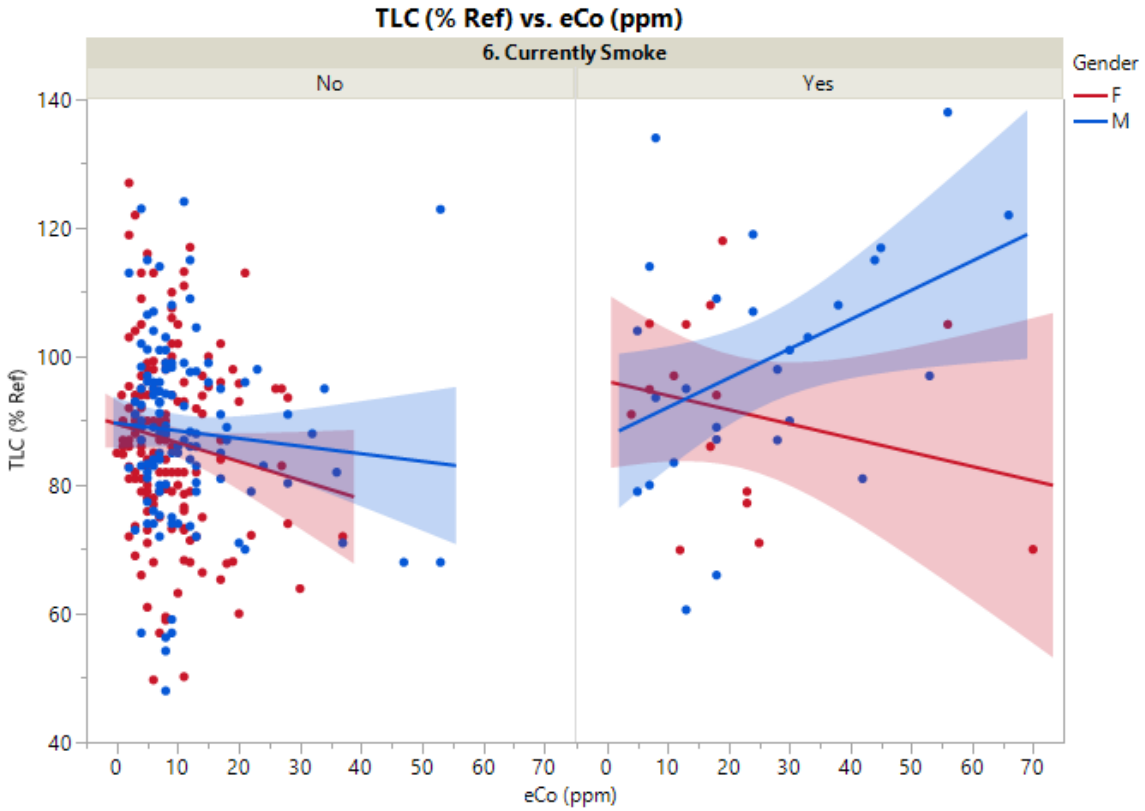



Figure 41. Relation between exhaled CO and TLC (% Ref) in nonsmokers and smokers of both gender.

There were no significant correlations between functional residual capacity (FRC) and eCO for nonsmokers, but positive correlations were found for smokers, both females and males (Fig. 42 and 43). With increased levels of eCO, FRC (% Ref) of the female smokers insignificantly increased, and that of the male smokers significantly increased. (Fig. 44).

A. Female nonsmokers Group

B. Female smokers Group

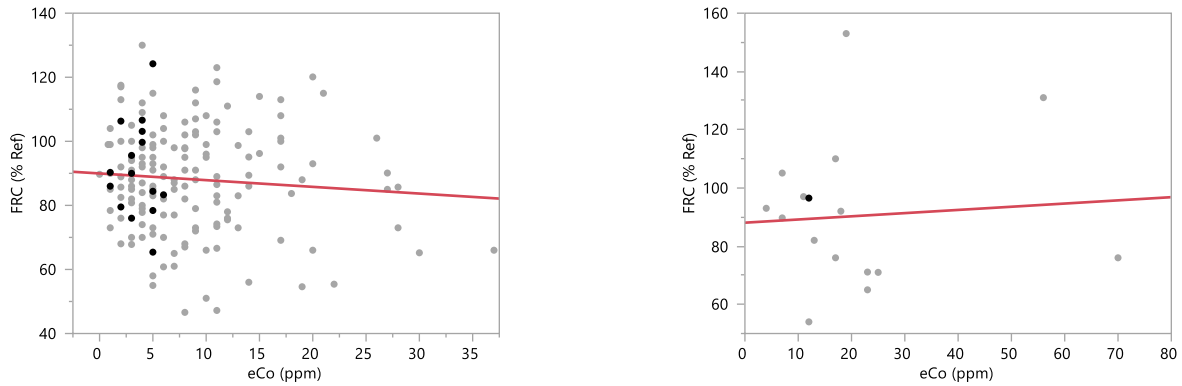



Figure 42. Relation between exhaled CO and FRC (% Ref) in ***female nonsmokers*** (r=-0.08; p=0.2897) (panel A) and ***female smokers*** (r=0.07; p=0.7592) (panel B). (Darker markers = Control group)

A. Male nonsmokers Group

B. Male smokers Group

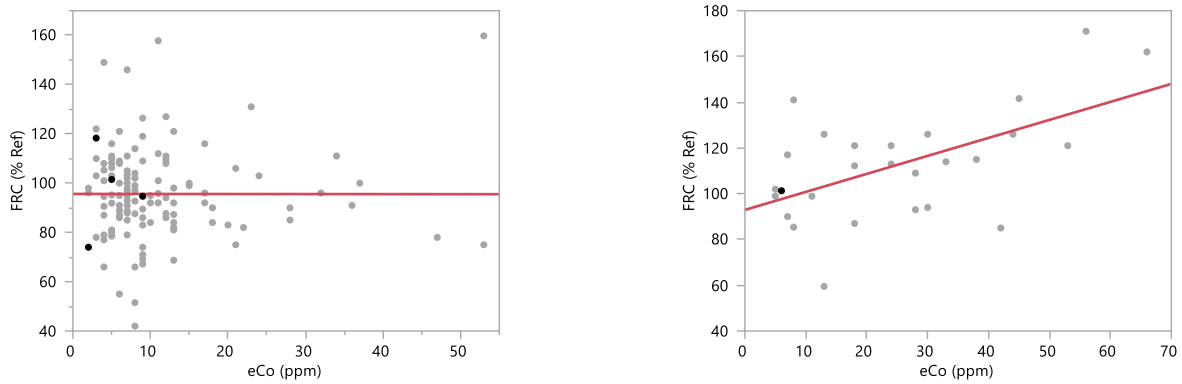



Figure 43. Relation between exhaled CO and FRC (% Ref) in ***male nonsmokers*** (r=0.06; p=0.9935) (panel A) and ***male smokers*** (r=0.56; p=0.0019) (panel B). (Darker markers = Control group)

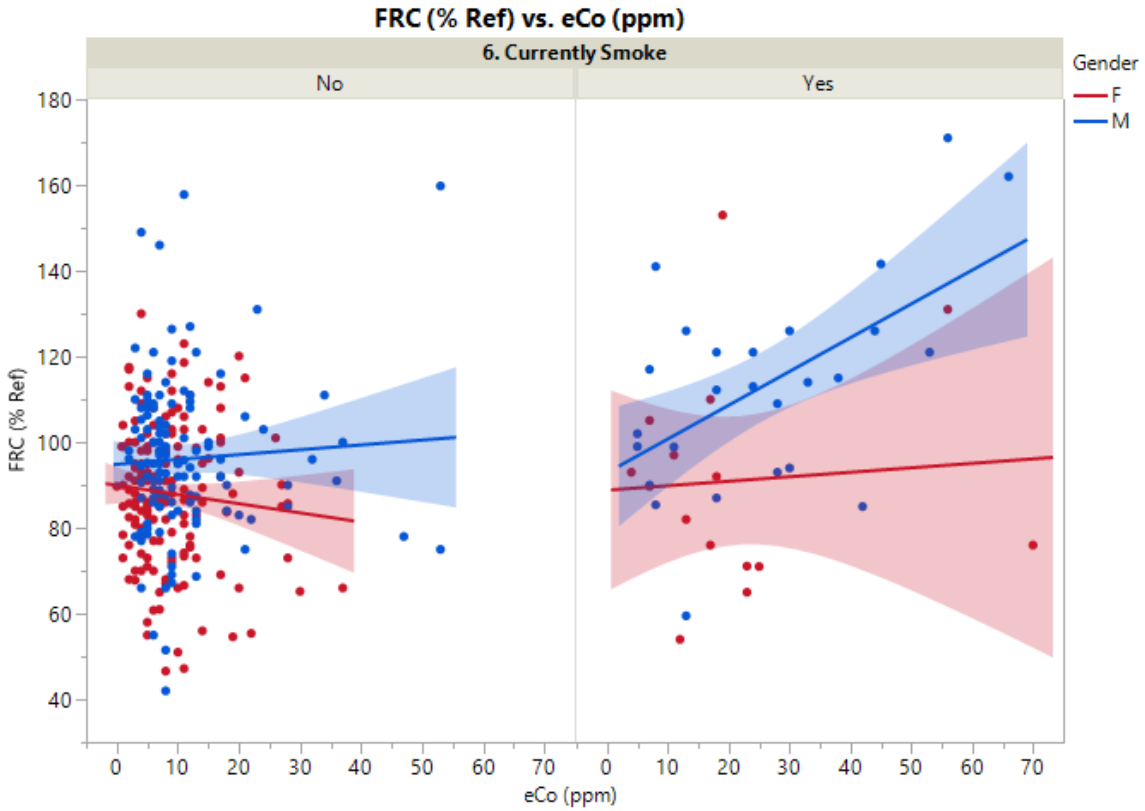
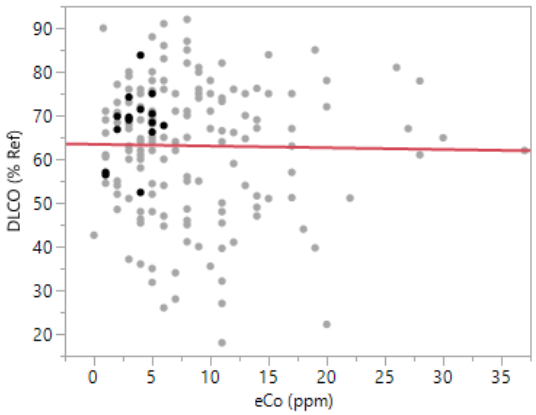



Figure 44. Relation between exhaled CO and FRC (% Ref) in nonsmokers and smokers of both gender.

***Relations between DLCO and eCO by Smoking Status***

Diffusing capacity of lungs for carbon monoxide (DLCO) test results also showed a trend toward opposite correlations for female and male smokers, a significant negative correlation for the female smokers (Fig. 45) and a non-significant positive correlation for the male smokers (Fig. 46). The relations between eCO and DLCO for both genders are shown in Figure 47.

A. Female nonsmokers Group




B. Female smokers Group

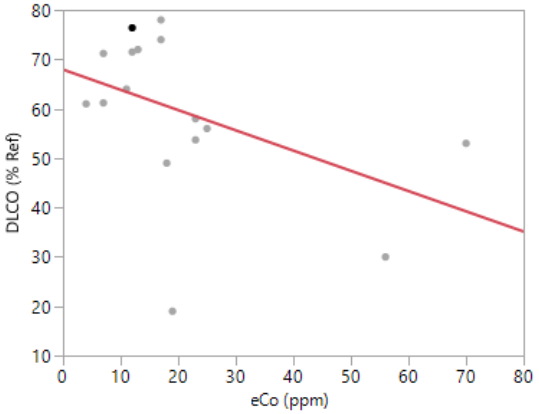
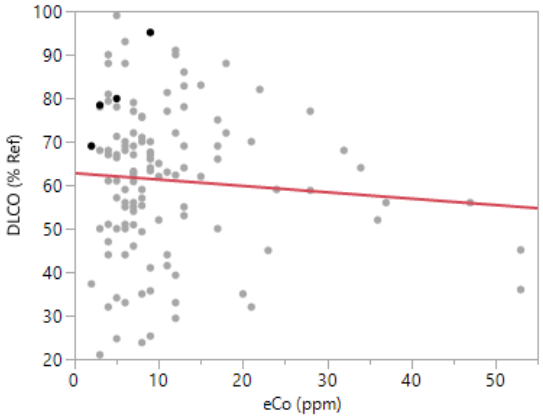



Figure 45. Relation between exhaled CO and DLCO (% Ref) in ***female nonsmokers*** (r=0.03; p=0.8005) (panel A) and ***female smokers*** (r=-0.45; p=0.0301) (panel B). (Darker markers = Control group)

A. Male nonsmokers Group




B. Male smokers Group

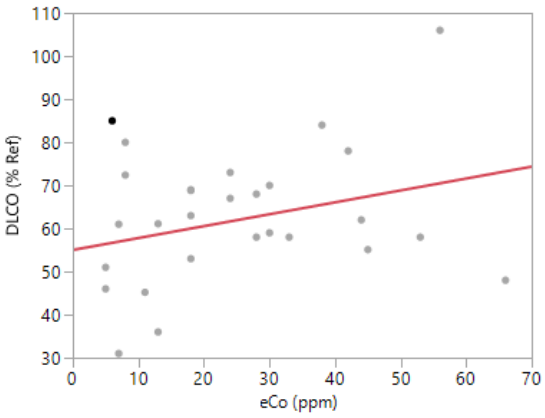



Figure 46. Relation between exhaled CO and DLCO (% Ref) in ***male nonsmokers*** (r=-0.08; p=0.2773) (panel A) and ***male smokers*** (r=0.33; p=0.3150) (panel B). (Darker markers = Control group)

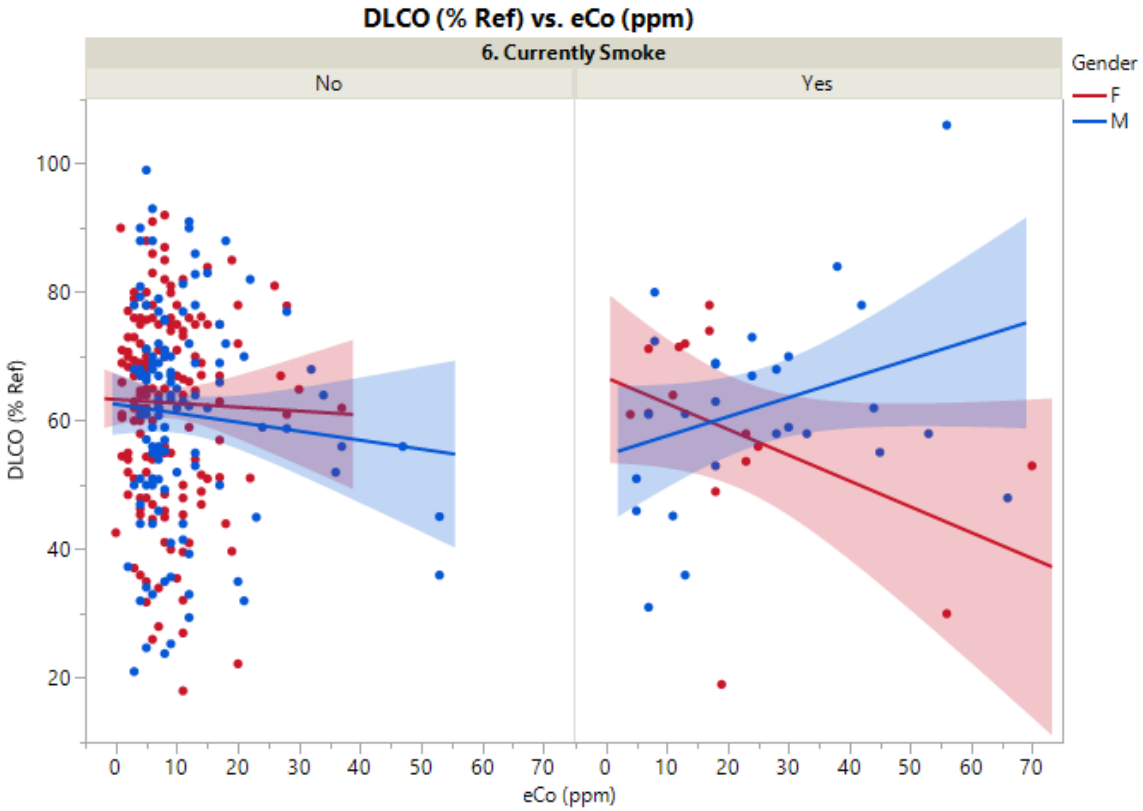



Figure 47. Relation between exhaled CO and DLCO (% Ref) in nonsmokers and smokers of both gender.

## Sensitivity and Specificity of eCO Test

Sensitivity and specificity are two main methods used to quantify the diagnostic accuracy of a test. Sensitivity is defined as the ability of a test to detect the disease when it is truly present and specificity as the ability of a test to identify when it is absent. In practice, clinicians aim for maximizing both sensitivity and specificity to increase the diagnostic ability of a test. Higher sensitivity is often considered desirable in a diagnostic test, and higher specificity is desirable in a screening test to rule out a disease (Mandrekar, 2010). Sensitivity is obtained by the ratio of true positives/ (true positives + false negatives), and specificity is obtained by the ratio of true negatives/ (true negative + false positives).

In this study, the eCO results are indicated as true positive if the eCO levels are higher than the cut-off points (positive) with abnormal PFT results (positive) and false negative if the eCO levels are lower than the cut-off points (negative) with abnormal PFT results (positive). The eCO results are indicated as true negative if the eCO levels are lower than the cut-off points (negative) with normal PFT results (negative) and false positive if the eCO levels are greater than the cut-off points (positive) with normal PFT results (negative) (Table 12).

Table 12. Indications of Test Results

| **PFT** | **eCO Test** | **Result** |
|---|---|---|
| Positive | Positive | True Positive |
| Positive | Negative | False Negative |
| Negative | Negative | True Negative |
| Negative | Positive | False Positive |

An obstructive defect is indicated by a forced expiratory volume in one second/forced vital capacity (FEV1/FVC) ratio with a reduced FEV1 which is defined as less than 70% based on data from the Third National Health and Nutrition Examination Survey (NHANES III) in adults (Johnson & Theurer, 2014). A forced expiratory flow in the region of 25-75% in FVC (FEF25-75%) less than 80% was also included as an obstructive defect (Table 13). A restrictive pattern is indicated by an FVC less than 80% with a normal or elevated FEV1/FVC ratio based on NHANES III data in adults or by a total lung capacity (TLC) less than 80%.

Table 13**.** PFT Criteria for Obstructive and Restrictive Lung Disease*

| **Obstructive Lung Disease:** | **Restrictive Lung Disease:** |
|---|---|
| FEV1/FVC < 70% with $FEV_1$ < 80%<br>$FEF_{25-75\%}$ < 80% | FVC < 80%<br>TLC < 80% |

*Based on data from the Third National Health and Nutrition Examination Survey (NHANES III) in adult.

To determine the best relationship between sensitivity and specificity, mean values of eCO were found by using the criteria for obstructive and restrictive lung disease based on adult data from the Third National Health and Nutrition Examination Survey (NHANES III) in adults. The mean values of eCO for female and male nonsmokers combined to meet the criteria were 9.7 ppm and 8.6 ppm for obstructive and restrictive lung disease, respectively (Table 14). Sensitivity and specificity were tested using the range of eCO from 6 to 9 ppm since the manufacturer of the carbon monoxide analyzer (MicroCO Meter) recommended to use 6 ppm as a normal level for nonsmokers (Table 15 and 17). To determine the eCO value that can be used to predict pulmonary abnormalities, the sensitivity and specificity values for each cut-off point are listed in Table 16 for obstructive lung diseases and in Table 18 for restrictive lung diseases. As the test results show in Figure in 48 and 49, sensitivity increased and specificity decreased when lower cut-off eCO values were used for obstructive and restrictive lung disease. Therefore, the highest sensitivity and the lowest specificity were achieved by using eCO level of 6 ppm.

Table 14. Mean Values of eCO for Obstructive and Restrictive Lung Diseases Determined by PFT

| **Criteria** | **eCO (ppm)** | **SD** |
|---|---|---|
| Obstructive Lung Disease (FEV1/FVC < 70% or $FEF_{25-75\%}$ < 80%) | 9.7 (n=186) | 8.7 |
| Restrictive Lung Disease (TLC < 80%) | 8.6 (n=21) | 4.9 |

SD = Standard Deviation

Table 15. Sensitivity and Specificity of eCO for **Obstructive Lung Disease**

**A.** Sensitivity and Specificity Test Results by using **eCO ≥ 9** for Obstructive Lung Disease. Disease prevalence of 67% (223/332), not including subjects with restrictive lung disease (n=32). The sensitivity is 48% (108/223) and specificity is 64% (70/109).

| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present (Abnormal PFT) | Absent (Normal PFT) | |
| Positive (eCO ≥ 9) | True positive (108) | False positive (39) | 147 |
| Negative (eCO < 9) | False negative (115) | True negative (70) | 185 |
| Total | 223 | 109 | 332 |

**B.** Sensitivity and Specificity Test Results by using **eCO ≥ 8** for Obstructive Lung Disease. Disease prevalence of 67% (223/332), not including subjects with restrictive lung disease (n=32). The sensitivity is 57% (127/223) and specificity is 58% (63/109).

| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present (Abnormal PFT) | Absent (Normal PFT) | |
| Positive (eCO ≥ 8) | True positive (127) | False positive (46) | 173 |
| Negative (eCO < 8) | False negative (96) | True negative (63) | 159 |
| Total | 223 | 109 | 332 |

**C.** Sensitivity and Specificity Test Results by using **eCO ≥ 7** for **Obstructive Lung Disease**. Disease prevalence of 67% (223/332), not including subjects with restrictive lung disease (n=32). The sensitivity is 64% (142/223) and specificity is 49% (53/109).

| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present (Abnormal PFT) | Absent (Normal PFT) | |
| Positive (eCO ≥ 7) | True positive (142) | False positive (56) | 198 |
| Negative (eCO < 7) | False negative (81) | True negative (53) | 134 |
| Total | 223 | 109 | 332 |

**D.** Sensitivity and Specificity Test Results by using **eCO ≥ 6** for Obstructive Lung Disease. Prevalence of 67% (223/332), not including subjects with restrictive lung disease (n=32). The sensitivity is 70% (156/223) and specificity is 39% (42/109).

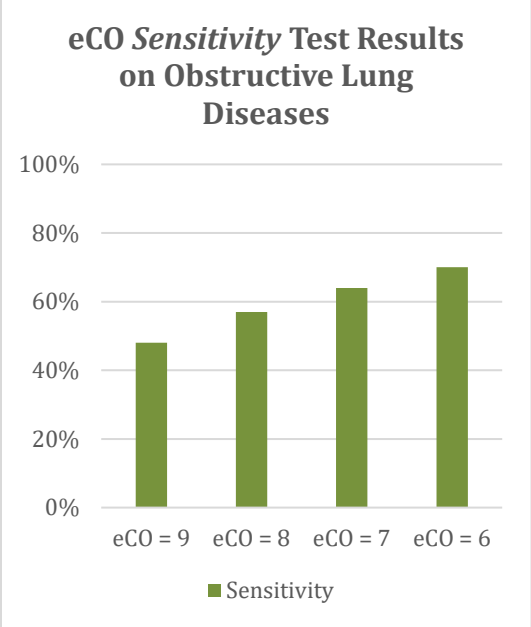
| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present (Abnormal PFT) | Absent (Normal PFT) | |
| Positive (eCO ≥ 6) | True positive (156) | False positive (67) | 223 |
| Negative (eCO < 6) | False negative (67) | True negative (42) | 109 |
| Total | 223 | 109 | 332 |

Table 16. Cut-Off Points of eCO for *Obstructive* Lung Diseases

| **eCO (ppm)** | **Sensitivity %** | **Specificity %** |
|---|---|---|
| **9** | 48 | 64 |
| **8** | 57 | 58 |
| **7** | 64 | 49 |
| **6** | 70 | 39 |

Abbreviation: eCO= exhaled carbon monoxide

A. Sensitivity




B. Specificity

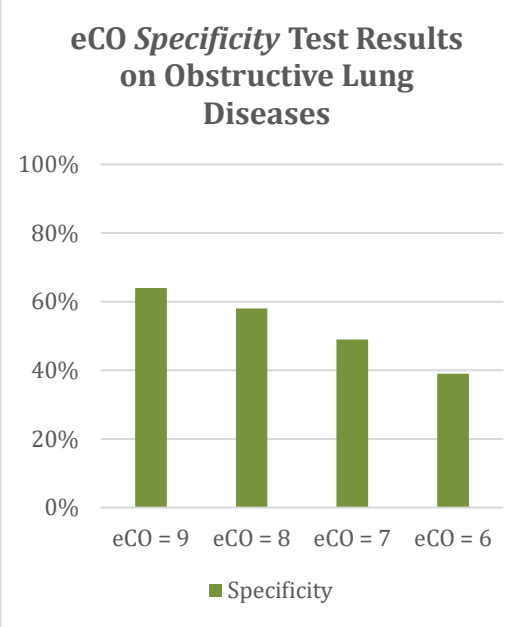



Figure 48. Sensitivity (panel A) and specificity (panel B) of eCO on obstructive lung disease.

Table 17. Sensitivity and Specificity of eCO for **Restrictive Lung Disease**

**A.** Sensitivity and Specificity Test Results by using **eCO ≥ 9** for Restrictive Lung Disease. Disease prevalence of 23% (32/141), not including subjects with obstructive lung disease (n=223). The sensitivity is 41% (13/32) and specificity is 64% (70/109).

| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present (Abnormal PFT) | Absent (Normal PFT) | |
| Positive (eCO ≥ 9) | True positive (13) | False positive (39) | 52 |
| Negative (eCO < 9) | False negative (19) | True negative (70) | 89 |
| Total | 32 | 109 | 141 |

**B.** Sensitivity and Specificity Test Results by using **eCO ≥ 8** for Restrictive Lung Disease. Disease prevalence of 23% (32/141), not including subjects with obstructive lung disease (n=223). The sensitivity is 47% (15/32) and specificity is 58% (63/109).

| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present (Abnormal PFT) | Absent (Normal PFT) | |
| Positive (eCO ≥ 8) | True positive (15) | False positive (46) | 61 |
| Negative (eCO < 8) | False negative (17) | True negative (63) | 80 |
| Total | 32 | 109 | 141 |

**C.** Sensitivity and Specificity Test Results by using **eCO ≥ 7** for Restrictive Lung Disease. Disease prevalence of 23% (32/141), not including subjects with obstructive lung disease (n=223). The sensitivity is 63% (20/32) and specificity is 49% (53/109).

| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present (Abnormal PFT) | Absent (Normal PFT) | |
| Positive (eCO ≥ 7) | True positive (20) | False positive (56) | 76 |
| Negative (eCO < 7) | False negative (12) | True negative (53) | 65 |
| Total | 32 | 109 | 141 |

**D.** Sensitivity and Specificity Test Results by using **eCO ≥ 6** for Restrictive Lung Disease. Disease Prevalence of 23% (32/141), not including subjects with restrictive lung disease (n=223). The sensitivity is 69% (22/32) and specificity is 39% (42/109).

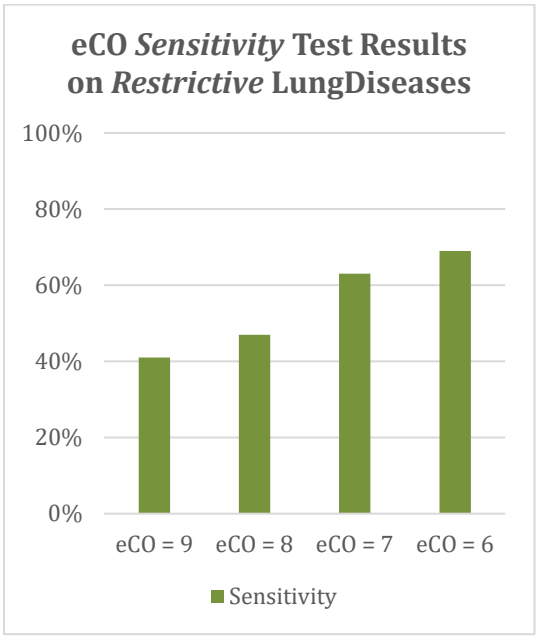
| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present (Abnormal PFT) | Absent (Normal PFT) | |
| Positive (eCO ≥ 6) | True positive (22) | False positive (67) | 89 |
| Negative (eCO < 6) | False negative (10) | True negative (42) | 52 |
| Total | 32 | 109 | 141 |

Table 18. Cut-off Points of eCO for *Restrictive* Lung Diseases

| **eCO (ppm)** | **Sensitivity %** | **Specificity %** |
|---|---|---|
| **9** | 41 | 64 |
| **8** | 47 | 58 |
| **7** | 63 | 49 |
| **6** | 69 | 39 |

Abbreviation: eCO= exhaled carbon monoxide

A. Sensitivity




B. Specificity

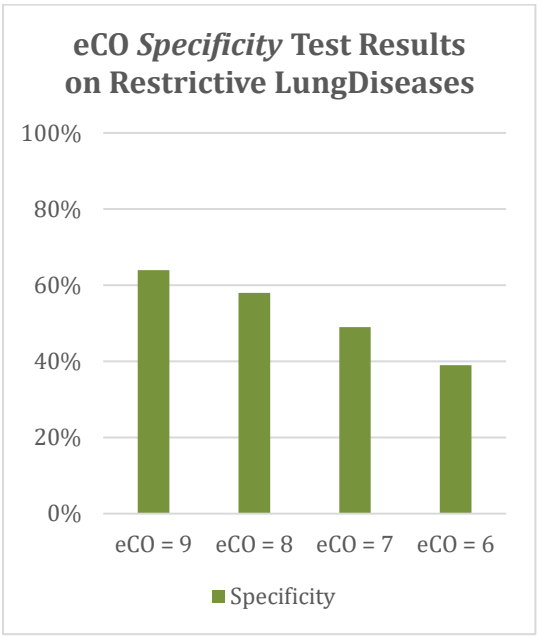



Figure 49. Sensitivity (panel A) and specificity (panel B) of eCO on restrictive lung disease.

# CHAPTER V: DISCUSSION

This chapter presents the discussion of the research results and integrates the principal study concepts of the correlational study and exploratory data analysis. The study limitations, implications for respiratory care practice, and recommendations for future research are also presented.

## Correlational Study

### Pulmonary Function Test (PFT)

#### Spirometry

In the obstructive lung disease group, mean values for spirometry tests revealed that obstructive ventilatory dysfunction and obstructive small airway disease were more severe in men than in women. Spirometry test results of subjects with restrictive lung diseases also revealed similarity with subjects with obstructive lung diseases. Females produced normal spirometry results on average while males had mild to moderate airflow limitation. In the group diagnosed with signs and symptoms, both females and males displayed a mild airflow limitation.

#### Lung Volumes

Although spirometry can measure inhaled and exhaled volumes, it cannot determine the total amount of air in the lungs at full inspiration (total lung capacity, TLC), the amount of air remaining in the lungs at the end of quiet (tidal) expiration (functional residual capacity, FRC), or the amount of air remaining after maximal expiration (residual volume, RV). Therefore, even though lung volume testing is not mandatory to identify an obstructive defect, it may, however, help to disclose underlying

disease and its functional consequences. For example, an increase in TLC, FRC, RV or the RV/TLC ratio above the upper limits of the normal range may suggest the presence of obstructive lung diseases, such as emphysema, asthma or other obstructive diseases.

The mean values of lung volume tests for both females and males in the three groups were in the normal ranges; total lung capacity (TLC), functional residual capacity (FRC), and residual volume (RV) were between 80% - 120% of the predicted values as shown in Table 3 and 4. However, male patients in the obstructive lung disease group produced a higher mean RV of 2.4 L (112%) compared with subjects of both females and males in other groups, indicating a tendency of air-trapping by increased obstruction. When their obstructive lung disease becomes severe, FRC, RV, and TLC tend to increase more as a result of airflow obstruction and decreased lung elastic recoil, causing increased air-trapping.

A restrictive ventilatory defect is characterized by a reduction in TLC below 80% of the predicted normal value with a normal FEV1/FVC (%Ref) since FEV1 and FVC tend to be reduced proportionally. The mean values of TLC for the three groups were greater than 80% of the predicted normal values, indicating that most of the subjects didn't have a restrictive defect although TLC of males with restrictive lung disease were in the low normal range, 84% of the predicted normal values.

**Diffusing Capacity of Lungs for Carbon Monoxide (DLCO)**

DLCO testing, in conjunction with spirometry and lung volumes assessment, may assist in diagnosing the underlying disease since spirometry and lung volumes can explain the mechanics of ventilation but do not address the gas-transfer function of the lung. In the DLCO test, with the use of carbon monoxide (CO) as a highly diffusible gas

and a surrogate for oxygen, a patient's ability to absorb alveolar gases can be estimated. When there are DLCO reductions, it may indicate pulmonary parenchyma and vascular disorders suggestive of interstitial lung disease, reductions in effective alveolar units that may be caused by the loss of surface areas such as in lung resection or emphysema, and anemia in spite of normal spirometry and lung volumes. Conversely, conditions resulting in an increased pulmonary blood volume, such as asthma or polycythemia, may cause an elevated DLCO (Evans & Scanlon, 2003). In this present study, mean values for the DLCO tests revealed that both females and males of the three groups had a mild to moderate diffusion defect. The lowest (55%) diffusing capacity was in the males of the restrictive lung disease group, and the highest diffusing capacity was in both females (64%) and males (64%) of the signs and symptoms group (Table 3 and 4).

**Exhaled Carbon Monoxide (eCO)**

As the results show (Table 3 and 4), eCO levels were significantly higher in patients than the control group (females: 4.1±0.6, males: 5.0±1.2). The highest mean eCO (15.3±1.8) was produced in males of the obstructive lung disease group, and the lowest mean eCO (8.4±0.9) in females of the restrictive lung disease group. This finding is consistent with the studies reported by Zayasu et al (1997), Horvath et al (1998b), Paredi et al (1999a) and Wood et al (2003). As expected and also reported by Montuschi et al (2001) and Kharitov et al (2002), the highest levels of eCO were found in patients diagnosed with emphysema with a mean eCO of 17.9 (Table 5).

Compared with the control group, patients in the restrictive lung disease group had a mild to a moderately increased level of eCO with a mean eCO concentration of 8.4 ppm in females and 12.3 ppm in males (Table 3 and 4). The highest levels of eCO were

found in patients being treated with chemotherapy with a mean eCO of 23.7 ppm (Table 6).

Exhaled CO levels were also mildly to moderately increased in patients admitted with signs and symptoms with a mean eCO concentration of 9.4 ppm in females and 12.1 ppm in males (Table 3 and 4). The highest levels of eCO were found in patients diagnosed with dyspnea on exertion with a mean eCO of 14 ppm (Table 7). This result suggests that acutely ill patients have a significantly higher CO concentration in exhaled air during exacerbations by increased expression of inducible HO-1.

Smoking causes an increase in exhaled carbon monoxide, and eCO levels were significantly higher, as expected, in current smokers compared to healthy nonsmokers as shown in Table 8, and the values of eCO in smoking and non-smoking subjects were similar to those of studies reported by Zayasu et al (1997), Montuschi et al (2001), and Deveci et al (2004). The highest eCO levels were produced by current female smokers in the signs and symptoms group with a mean of 37.4 ± 10.9 ppm and the obstructive lung disease group of male subjects with a mean of 27.5 ± 5.6 ppm. These results reflect that the increased eCO levels are directly proportional to the concentration of carboxyhemoglobin (COHb) in the blood of the subjects since habitual smokers may have COHb values typically between 5-15% or higher than nonsmokers (Ryter and Choi, 2013). Furthermore, the present study found that eCO levels of former smokers were similar to those of nonsmokers, indicating that the eCO was produced from endogenous sources rather than from smoking.

Use of anti-inflammatory drugs are supposed to decrease inflammation; however, as shown in Table 10, the results were not quite as expected. Female subjects of the two

groups, restrictive lung disease and signs and symptoms, who were receiving anti-inflammatory drugs produced lower eCO levels, as expected than females who were not using the drugs. However, eCO levels were higher (12.3 ± 1.8 ppm) in female subjects of the obstructive disease group who were receiving anti-inflammatory drugs than those of female subjects who were not using the drugs (8.8 ± 1.0 ppm). These findings contradict the conclusions of Zayasu and coworkers (1997) who studied eCO in asthmatic patients receiving or not receiving inhaled corticosteroids and compared it to CO levels in nonsmoking and smoking healthy control subjects.

On the other hand, the results of eCO levels of male subjects were opposite from the results of female subjects. eCO levels of male subjects who were receiving anti-inflammatory drugs were higher than those of males who were not receiving the drugs in the restrictive lung disease group (25.0 ± 14.0 ppm) and signs and symptoms group (18.3 ± 7.1 ppm) although it may not be statistically significant since there were only 3 and 6 male subjects in the two groups, respectively.

Two main reasons might be associated with these findings. First, eCO level might be higher than expected in patients receiving anti-inflammatory drugs because the patients were put on the drugs recently due to acute lung diseases. It may indicate that females have a tendency to develop chronic restrictive lung diseases and acute obstructive lung diseases while males have the opposite situations. Second, although it is known that inhaled steroids reduce inflammation, the patients might have other systemic inflammatory diseases, such as systemic lupus erythematosus (SLE) or arthritis, at the time of this study, leading to increased eCO levels.

**Relations between PFT and eCO**

The present study found that there were negative correlations between eCO levels and the spirometry test. FVC, FEV1, FEV1/FVC, and $FEF_{25-75\%}$ decreased with elevated concentrations of eCO levels (Fig. 13-20). This result is consistent with the studies reported by of Zayasu et al (1997), Zanconato et al (2002), and Wood et al (2003), and the increased production of eCO suggests that expression of HO-1 in epithelial cells of the airway is increased in airway inflammation.

Lung volumes and eCO levels showed the opposite correlation between females and males. The female group had a negative correlation between the measurements of lung volume test and eCO levels while the male group had a positive correlation (Fig. 21-24). It's known that total lung capacity (TLC) and functional residual capacity (FRC) are usually reduced in restrictive disorders, such as interstitial lung disease, and they may be normal or may be elevated (hyperinflated) due to air trapping in obstructive disorders, such as COPD. In this study, both TLC and FRC of the female group decreased with increasing eCO while those of the male group increased. The results suggest that when a respiratory disease develops, females tend to have a restrictive lung pattern while males tend to have an obstructive lung pattern.

DLCO of female subjects decreased with increased levels of eCO while DLCO of male subjects had no significant association with eCO (Fig. 25 and 26). Since DLCO estimates a patient's ability to absorb alveolar gases, the DLCO reductions in female subjects reflect compromised oxygen uptake by pulmonary parenchyma and vascular disorders in either obstructive or restrictive disorders. In contrast, the lack of an association in male subjects may indicate that the patients had conditions that cause

increased in hemoglobin, such as polycythemia, a common adaptation to lung diseases to enhance oxygen uptake.

**Sensitivity and Specificity of eCO as a Diagnostic Test**

Diagnostic test results should be able to classify patients into two groups according to the presence or absence of abnormalities. The terms positive and negative are used to refer to the presence or absence of the condition of interest. Sensitivity is the proportion of true positives that are correctly identified by the test. Specificity is the proportion of true negatives that are correctly identified by the test (Altman & Bland, 1994). To determine the potential for using the eCO test for diagnostic screening it is necessary to know how good the test is at predicting pulmonary abnormalities.

Exhaled CO values greater than the cut-off points are defined as inflammatory status. This present study found that the cut-off point for eCO concentration for obstructive or restrictive lung diseases was 6 ppm for the best relationship between sensitivity and specificity, and this result is consistent with other research (Zayasu et al, 1997; Paredi et al, 1999a, 2000). By using an eCO of 6 ppm as a cut-off point, the highest sensitivities, 70% for obstructive lung diseases (Table 16) and 69% for restrictive lung disease (Table 18), were yielded. Specificity, however, was lower by using an eCO of 6 ppm, 39% for both disease types. The specificity of 39% was the same for the obstructive and restrictive lung disease groups since the number of patients who had normal PFT results were the same from the group of participants and the specificity was calculated as true negative/ (false positive + true negative). The lower specificity of 39% (42/67+42) does not necessarily invalidate the use of eCO since, although the 67 patients were false positive, which is eCO≥6 with a normal PFT, it might suggest that the

pulmonary function test was not as sensitive as using eCO to detect the presence of inflammation. The findings of this study suggest that eCO measurements can improve the diagnostic accuracy of lung diseases when associated with clinical examination since the patients who were false positive had clinical symptoms such as shortness of breath, dyspnea, or chronic cough.

## Limitations of the Study

Limitations for this research study were identified pertaining to the sample characteristics, variables that can be used to identify inflammation, and the limited information on drugs that patients were taking. The current study had a convenience to use patients who referred to the pulmonary lab, and the majority of the participants were elderly, mean age of 62.5 years for females and 59.5 years for males. Using the elderly subjects as a sample group may have contributed to the confounding results since they tend to have multiple medical issues in which the sources of inflammation can be unclear. Another limitation pertaining to the sample characteristics is that most subjects were overweight with a mean BMI $\geq$ 27.5 kg/m$^2$ for females and 29.7 kg/m$^2$ for males which might hamper generalizability of the results to normal weight people. Obesity affects the respiratory system by significantly interfering with respiratory function due to the changes in physiological mechanisms such as fat deposition in the chest wall, abdomen, and upper airways, and also via systemic inflammation. Consequently, the findings' generalizations are limited to groups that share the same characteristics represented in this sample.

Having no information on some variables, including eosinophil count or C-reactive protein, which can be used to identify inflammation is also a limitation of the

research study. The airway inflammation can be assessed by the measurement of blood or sputum eosinophils that increase in asthma (Pizzichini et al., 1997) or COPD (Saha et al, 2006). The measurement of C-reactive protein is also an exquisitely sensitive systemic marker of inflammation and tissue damage (Ridker, 2000). Having information on such measurements would be a powerful tool to identify the sources of inflammation.

Another limitation is having insufficient information on medications the patients were taking, including anti-inflammatory drugs. The data on drug information was mostly collected by self-administered questionnaire, and in some situations, the patients were not able to recall the names of the drugs they were taking, which can consequently influence data accurateness. Furthermore, because both pulmonary function and eCO levels can change over time as a result of treatment, the participants might have had low eCO levels during the data collection whereas their eCO levels might have been increased at other times.

## Implications for Respiratory Care Practice

This study intended to explore the possible associations between pulmonary function testing (PFT) and eCO levels. Findings from this dissertation research highlight the importance of utilizing the eCO measurement for assessing inflammation. PFT plays an essential role in the management of pulmonary diseases and is used extensively in clinical settings. PFT is relatively simple and non-invasive, and the test provides objective lung function assessments that clinicians can correlate with symptoms such as shortness of breath or dyspnea. PFT yields reproducible and quantitative results that allow clinicians to monitor the course of a disease and the effectiveness of treatments.

However, despite its clinical importance, PFT is not well utilized by primary care physicians, and this problem is partially due to a lack of established indications for PFT. Measurement of eCO levels may be a simple method of detecting inflammation in the respiratory system and of assessing anti-inflammatory treatments. Many pulmonary diseases such as asthma, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis involve chronic inflammation and oxidative stress. However, it is difficult to detect these diseases before respiratory dysfunction occurs because of their progression is not measured directly in routine clinical practice due to the difficulties in monitoring inflammation.

Measurement of eCO may assist in the diagnosis of pulmonary diseases and assessment of their severity. Because these techniques are non-invasive and inexpensive, eCO monitoring can be used repeatedly to provide information about the response to treatments and thus measuring the effectiveness of therapy. Further evaluation of its use in hospital and family practice settings as well as personal monitoring by patients seems warranted.

## Recommendations for Future Respiratory Care Research

Exhaled breath analysis has enormous potential as a noninvasive means of monitoring airway inflammation. Exhaled breath content contains biomarkers, such as carbon monoxide (eCO), nitric oxide (NO), and hydrocarbons, of respiratory function and can be used to detect inflammation in the respiratory system (Kharitonov, 2001). Therefore, future studies should focus on the effectiveness of using eCO to monitor the effectiveness of anti-inflammatory treatments and the relation between eCO and other direct inflammatory markers. More studies are needed in characterizing the biomarkers

in an exhaled breath so that a disease may have a characteristic profile or fingerprint of different markers that may be diagnostic.

## Summary

Respiratory disorders are a considerable cause of morbidity and mortality in the United States, and many lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and interstitial lung disease, involve chronic inflammation and oxidative stress. However, the diseases often are not diagnosed and are not treated efficiently in routine clinical practice for a variety of reasons, including difficulty in monitoring inflammation. In many cases, inflammation of the respiratory tract may have been present in patients well before pulmonary function impairment is diagnosed.

Pulmonary function testing (PFT) plays an essential role in the management of respiratory dysfunction by providing objective lung function assessments that the clinician can correlate with patients' symptoms such as shortness of breath or dyspnea. By utilizing PFT, primary care physicians can make early diagnosis and can plan treatments and monitor the responses to treatments effectively.

However, PFT is not well utilized by primary care physicians because there are no well-established indicators for PFT before respiratory dysfunctions occur in patients. Therefore, when patients are sent to a pulmonary function testing lab because of their symptoms, it's often too late to alter the respiratory dysfunction, and thus there is a need to establish a standardized indicator for PFT so that primary care physicians can make a timely diagnosis and offer effective treatment plans to patients.

Since it has been reported that exhaled carbon monoxide (eCO) is significantly increased in patients with chronic lung diseases such as asthma and COPD, this study intended to explore the possible associations between PFT measures and eCO levels to establish a cut-off point of eCO as an indicator for PFT. The results of this present study found that there is a negative correlation between eCO levels and spirometry test measurements. Forced Vital Capacity (FVC), Forced Expiratory Volume FEV1, FEV1/FVC, and Forced Expiratory Flow ($FEF_{25-75\%}$) decreased with elevated concentrations of eCO levels.

The relations between lung volume measurements and eCO levels showed different results for females and males. The female group had a negative correlation between lung volume test and eCO levels while the male group had an insignificant positive correlation. The results of DLCO testing and eCO levels also showed the opposite correlation between females and males. DLCO of female subjects markedly decreased with increased levels of eCO while DLCO of male subjects insignificantly increased. The increased production of eCO in patients with pulmonary diseases suggests that expression of HO-1 in epithelial cells of the airway is increased due to airway inflammation.

The cut-off points of eCO were examined to determine the inflammatory status, and this present study found that the optimal cut-off point of eCO to indicate possible obstructive or restrictive lung diseases was 6 ppm for the best relationship between sensitivity and specificity.

In conclusion, this study found that the levels of eCO elevate when inflammation occurs in the lungs. Therefore, eCO measurement could serve as a practical biomarker to

identify inflammation and as an indicator for PFT. On the other hand, eCO may be of limited use as a diagnostic tool in patients with complex diseases such as COPD especially in the presence of confounding variables such as continued smoking and environmental carbon monoxide as a pollutant.

# APPENDIX A: UNIVERSITY OF HAWAI'I AT MĀNOA – HUMAN SUBJECTS PROGRAM APPROVAL LETTER

Office of Research Compliance
Human Studies Program

UNIVERSITY of HAWAI'I SYSTEM

August 30, 2016

Jun Eun Kim
Respiratory Physiology

Subject: CHS #24102 - "Evaluating Endogenous Carbon Monoxide (CO) Production as an Indicator for Pulmonary Function Testing (PFT)"

Dear Jun Eun Kim,

The University of Hawaii (UH) Human Studies Program has received notice that an application for human subjects review and approval of the study identified above has been submitted to the Queen's Medical Center IRB (QMC).

Under the IRB Authorization Agreement executed between QMC and UH, UH cedes authority to QMC for human subjects review of your study. Please provide the Human Studies Program with the following:

- A copy of the communication you receive from QMC documenting approval or disapproval of your study;
- A copy of the QMC-approved consent form(s), if applicable;
- Any unanticipated problems & serious adverse events; and
- Notification of changes in the study status (for example, when it is complete, suspended or withdrawn).

You may submit the required information and documents to the Human Studies Program via email to uhirb@hawaii.edu.

Please be aware that you are responsible for ensuring compliance with the QMC's determinations pertaining to this study as well as its policies and procedures for seeking approval of study modifications, applying for continuing review, reporting unanticipated problems, etc. You should contact QMC directly if you have questions about these requirements.

Thank you for your cooperation. Please contact our office at 808-956-5007 if you have any questions.

Sincerely,

Denise A. Lin-DeShetler, MPH, MA
Director

1960 East-West Road
Biomedical Sciences Building B104
Honolulu, Hawai'i 96822
Telephone: (808) 956-5007
Fax: (808) 956-8683

## APPENDIX B: THE QUEEN'S MEDICAL CENTER – RESEARCH & INSTITUTIONAL REVIEW COMMITTEE APPROVAL LETTER

**THE QUEEN'S MEDICAL CENTER**
RESEARCH & INSTITUTIONAL REVIEW COMMITTEE

University Tower 5th Floor ▪ 1301 Punchbowl Street ▪ Honolulu, Hawaii 96813 ▪ Phone (808) 691-4512 ▪ FAX: (808) 691-7897

Date: November 21, 2016

To: Jung Eun Kim, MS
4303 Diamond Head Road, Health Sciences Dept Rm 122
Honolulu, Hawaii 96816

***EXPEDITED NEW APPROVAL***

Re: **RA-2016-310** **Evaluating Endogenous Carbon Monoxide (CO) Production as an Indicator for Pulmonary Function Testing (PFT)**

On behalf of the Research and Institutional Review Committee (RIRC) of The Queen's Medical Center, I am pleased to grant you expedited approval for the above referenced application assigned RA-2016-310. This is an identification number and should be used on all correspondence and documentation related to the study.

| | | |
|---|---|---|
| APPROVAL DATE: | **11/21/2016** | Expedited approval. General review to follow. |
| EXPIRATION DATE: | **10/31/2017** | Application for its continued renewal must be made at least two months prior to the expiration date. |
| ADVERSE REACTIONS: | | All serious adverse events must be reported to the RIRC within 3-5 working days. |
| MODIFICATIONS: | | Any change (minor or major) to the study must have prior RIRC approval. |
| DOCUMENTATIONS: | | All correspondence relating to this study must be kept on file; all relevant papers are subject to federal inspection. |

COMMENTS:

**Expedited approval for study under Category 4 (collection of data through noninvasive procedures routinely employed in clinical practice). This study will enroll 500 patients with pulmonary disease and 50 patients without pulmonary disease who are already scheduled for routine Pulmonary Function Testing. Exhaled carbon monoxide measurement prior to the routine PFT, and an anonymous health questionnaire will be administered. All data will be anonymized. Approval includes**

- **Protocol version 21Nov2016,**
- **Study Information Sheet version 18Oct2016,**
- **Patient Health Questionnaire with Data Collection Form version 18Oct2016,**
- **Recruitment/Enrollment Script version 18Oct2016, and**
- **waiver of documenting informed consent.**

All portable devices (including laptops) used or taken off QMC campus containing any protected health information must have full disk encryption (not just file encryption). If there are questions regarding products please contact QMC Help Desk at 691-4357.

QUESTIONS: Please contact the Research Regulatory Office at (808) 691-4512.

Yours sincerely,

Michael Meagher, MD, FACR
Chairman, Research and Institutional Review Committee

cc: file
UH HSP

## APPENDIX C: HUMAN SUBJECTS STUDY INFORMATION SHEET



### *Study Information Sheet*

**Title**: Evaluating Endogenous Carbon Monoxide (CO) Production as an Indicator for Pulmonary Function Testing (PFT)

**Principal Investigator**: Jung Eun Kim, MS, RRT, CPFT
University of Hawaii, Kapi'olani Community College
4303 Diamond Head Road
Health Sciences Department, Room 112
Honolulu, HI, 96816
Telephone: (808) 734-9243

**Co-Investigators**: Roger Yim, MD (Queen's Medical Center)
C. Alan Titchenal, PhD (University of Hawaii)



**Purpose of the study**: This study tries to find out whether measuring the exhaled (breathing out) carbon monoxide (eCO) can be used as an early sign for the need of a Pulmonary Function Test (PFT) which is often an important part in the diagnosis and treatment of severe respiratory (breathing) problems. We are planning to enroll about 550 volunteers into this study. Most of the volunteers (about 500) would have some kind of breathing problems, while about 50 would take part as controls (no breathing problems).

**Study procedure**: If you agree to participate, the following will be your part in the study:

- You will be asked to fill out a health questionnaire.
- You will be asked to inhale (breath in) deeply and hold your breath for 8-10 seconds and exhale (breath out) into a disposable mouthpiece of a carbon monoxide monitor, similar to an alcohol breathalyzer.

After this you will have your PFT, which was scheduled by your doctor for your routine care and is the actual reason why you are here.

- While doing the breathalyzer test (eCO) and the PFT, the researchers will also record (write down) some of the test results for this study. None of these data will have your name or other identifying information attached.
- Your participation is voluntary, and you can stop for any reason, at anytime by informing the person who is performing the tests or Jung Eun Kim at 808-734-9243 and it will not make any difference to the care that you will receive today or in the future.

9243 and it will not make any difference to the care that you will receive today or in the future.

**Potential risks to study participants**:

No risk is associated with the intended use of a carbon monoxide monitor (breathalyzer). The measurement of your exhaled air is non-invasive and similar to an alcohol breathalyzer test.

**Potential benefits to study participants**:

There will likely not be any direct benefit for you while participating in this study, but results from this research could benefit future patients due to better diagnostic procedures.

**Confidentiality**:

Your name or other identifying information will not be recorded on any study documents except the date of your pulmonary function test and your age (in years). Date information is needed to find out whether the weather condition (like vog or cold weather) may affect the results of your testing. Your age information is needed for the analysis of the results. For the purpose of keeping track of the number of study volunteers, your questionnaire and the breathing test data (collected on the back side of the questionnaire) will receive a study-specific identifier (number/letter code), which can't be linked back to your name. Because we are planning to enroll about 500 volunteers, and your data will be analyzed and presented together (in aggregate), the resulting information will be anonymous.

If you have any questions, you can reach Jung Eun Kim at 808-734-9243.

If you can't get into contact with Jung Eun Kim, or if you have any questions about your rights as a volunteer in this study, you can also contact the Research and Institutional Review Committee of the hospital, 1301 Punchbowl Street, Honolulu, Hi 96813 or 808-691-4512.

# APPENDIX D: HEALTH QUESTIONNAIRE AND DATA COLLECTION FORM



**Health Questionnaire**

1. Do you have a shortness of breath? Yes ___ No ___
   If yes, how long have you had shortness of breath? ____ years _____ months
   What causes your shortness of breath? _____
2. When was the last time you had wheezing? Never ____ Within the last week ____
   Last month ____ Last year ____
3. Do you have chronic sputum production? Yes ___ No ___
4. Do you have chronic cough? Yes ___ No ___
5. Any history of lung diseases? Yes ___ No ___
   If yes, what diseases did you have? _____
6. Do you currently smoke? Yes ___ No ___

A) If yes, what do you smoke? Cigarettes ___ Cigars ___ e-cigs ___
   Others: _____
   How much do you smoke per day? _____
   How long have you been a smoker? _____ years _____ months

B) If you quit, how long ago? _____ years ago _____ months ago
   How much did you smoke per day? _____
   How long did you smoke before quitting? _____ years _____ months

7. Are you taking any medications? Yes ___ No ___
   If yes, what are the names of medications?
   _____
8. Have you been told that you have anemia? Yes ___ No ___
9. Have you been told that you are iron deficient? Yes ___ No ___
10. Are you: vegetarian? Yes ___ No ___ vegan? Yes ___ No ___
    If no, number of times per week you usually eat the following:
    Beef _____ Pork _____ Poultry _____ Fish _____
11. Are you on diet to lose weight? Yes ___ No ___
12. Have you donated blood? Yes ___ No ___
    If yes, when was the last time you donated blood? _____
13. Did you exercise this morning? Yes ___ No ___
    If yes, what type of exercise did you do? _____

**THIS PAGE WILL BE FILLED IN BY RESEARCH PERSONNEL**

14. Diagnosis (per referral slip): _____
15. Gender: M _____ F _____
16. Age: _____ years old
16. Height: _____ inches
17. Weight: _____ lbs
18. Ethnicity (per patient report):
19. Body Type (per investigator impression): Normal _____ Muscular _____
20. Visit/Test date:

**Data Collection:**

| Subject # | eCO (ppm) | FVC (Actual Value /% Ref) | FEV1 (Actual Value /% Ref) | $FEF_{25-75\%}$ (Actual Value /% Ref) | FEV1/FVC Actual Value (%) | TLC (Actual Value /% Ref) | FRC (Actual Value /% Ref) | RV (Actual Value /% Ref) | DLCO (Actual Value /% Ref) | Hb (g/dL) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |
| | | | | | | | | | | |

## APPENDIX E: RECRUITMENT SCRIPT

RA-2016-310

RECRUITMENT /ENROLLMENT SCRIPT

Volunteers for this study will be recruited by members of the research team, who are also staff of the QMC Pulmonary Lab. Recruitment will be through direct contact with patients who have been referred to the QMC Pulmonary Lab by their physicians for a PFT.

The recruitment message text is as follows:

*Would you be interested in participating in a research project?*

*Thank you for your interest in our study. This study is being conducted by Jung E. Kim, a graduate student in the Department of Anatomy, Biochemistry, and Physiology at The University of Hawaii working on his doctoral degree. His supervising professor is C. Alan Titchenal, PhD, and the co-investigator is Roger Yim, MD, the Medical Director of the Pulmonary Lab. We are mainly looking for individuals who have symptoms of acute or chronic lung diseases such as asthma and Chronic Obstructive Pulmonary Disease and are scheduled for pulmonary function testing. We are also looking for some volunteers who have no breathing problems, but could serve as control subjects in this study. As part of the study, we will measure the concentration of carbon monoxide in your exhaled breath before your pulmonary function test today. This will involve exhaling into a device similar to what is used for alcohol breath tests (breathalyzer). If you are still interested to participate, please read the Study Information sheet and let us know if you have any questions. If you are not interested in taking part in this study, it will not make any difference to the care that you will receive today or in the future.*

OCT 21 2016
RESEARCH REGULATORY OFFICE

QMC INSTITUTIONAL REVIEW COMMITTEE
DATE 11.21.16
EXPIRES 10.31.17
APPROVED

Version 18Oct2016

Protocol: Evaluating Endogenous Carbon Monoxide ...

APPENDIX F: PFT AND eCO RESULTS OF ENTIRE PARTICIPANTS

## APPENDIX F: PFT AND eCO RESULTS OF ENTIRE PARTICIPANTS

Table F.1. PFT Results of the Entire Participants

| | | **Control** (N=21) | | **Subjects with Obstructive Lung Disease** (N=137) | | **Subjects with Restrictive Lung Disease** (N=50) | | **Subjects with Signs & Symptoms** (N=156) | |
|---|---|---|---|---|---|---|---|---|---|
| Gender | | F (N=16) | M (N=5) | F (N=75) | M (N=62) | F (N=29) | M (N=21) | F (N=82) | M (N=74) |
| **Spirometry (Mean Values)** | | | | | | | | | |
| FVC | L (% Ref) | 3.3 (92) | 5.1 (96) | 2.4 (87) | 3.3 (77) | 2.7 (94) | 3.2 (80) | 2.5 (88) | 3.8 (85) |
| | p-Value | | | 0.3322 | **0.0187** | 0.8588 | 0.0636 | 0.3737 | 0.1116 |
| FEV1 | L (% Ref) | 3.0 (92) | 4.4 (102) | 1.8 (80) | 2.3 (66) | 2.1 (93) | 2.3 (73) | 2.0 (87) | 2.9 (81) |
| | p-Value | | | **0.0356** | **0.0023** | 0.7309 | **0.0134** | 0.2635 | **0.0165** |
| FEF25-75% | L/s (% Ref) | 3.6 (94) | 5.4 (119) | 1.4 (65) | 1.6 (49) | 1.9 (88) | 1.8 (58) | 1.7 (75) | 3.5 (69) |
| | p-Value | | | **0.0005** | **0.0014** | 0.3612 | **0.0022** | **0.0018** | **0.0091** |
| FEV1/ FVC | % | 89 | 87 | 72 | 67 | 78 | 70 | 78 | 76 |
| | p-Value | | | **<0.0001** | **0.0008** | **<0.0001** | **0.0025** | **<0.0001** | **0.0049** |
| **Lung Volumes (Mean Values)** | | | | | | | | | |
| TLC (L) | L (% Ref) | 4.5 (91) | 6.4 (93) | 4.2 (88) | 5.8 (90) | 4.2 (87) | 5.4 (84) | 4.2 (87) | 6.0 (92) |
| | p-Value | | | 0.4989 | 0.4235 | 0.2882 | 0.1521 | 0.1535 | 0.7322 |
| FRC (L) | L (% Ref) | 2.5 (92) | 3.3 (98) | 2.8 (90) | 3.4 (101) | 2.3 (85) | 3.2 (95) | 2.4 (88) | 3.3 (99) |
| | p-Value | | | 0.7909 | 0.8535 | 0.3124 | 0.7203 | 0.3538 | 0.8019 |
| RV (L) | L (% Ref) | 1.1 (87) | 1.4 (76) | 1.7 (87) | 2.4 (112) | 1.5 (80) | 2.1 (92) | 3.6 (83) | 2.2 (105) |
| | p-Value | | | 0.9914 | 0.0596 | 0.6318 | 0.4741 | 0.5229 | 0.0830 |
| **DLCO (Mean Values)** | | | | | | | | | |
| DLCO | L /s/mm Hg (% Ref) | 20.4 (68) | 31.8 (82) | 14.7 (63) | 18.9 (60) | 13.8 (57) | 15.9 (55) | 15.4 (64) | 20.7 (64) |
| | p-Value | | | 0.3125 | **0.0102** | **0.0108** | **0.0016** | 0.3675 | **0.0071** |
| Hb (g/dL) | | | | 13.1 | 14.4 | 12.7 | 12.1 | 13.1 | 14.5 |

Table F.2. Exhaled CO levels in the different diagnosis group and their characteristics

| | **Control** (N=21) | | **Subjects with Obstructive Lung Disease** (N=137) | | **Subjects with Restrictive Lung Disease** (N=50) | | **Subjects with Signs & Symptoms** (N=156) | |
|---|---|---|---|---|---|---|---|---|
| Gender | F (N=16) | M (N=5) | F (N=75) | M (N=62) | F (N=29) | M (N=21) | F (N=82) | M (N=74) |
| Age (mean) (yr) | 27.6 (SD=6.2) | 35.0 (SD=11.4) | 63.9 (SD=13.1) | 60.4 (SD=14.7) | 62.1 (SD=16.5) | 68.6 (SD=13.1) | 61.5 (SD=14.4) | 56.9 (SD=14.8) |
| BMI (mean) ($kg/m^2$) | 24.4 (SD=4.6) | 27.4 (SD=2.5) | 27.7 (SD=10.1) | 30.5 (SD=7.9) | 25.3 SD=5.6) | 28.7 (SD=6.1) | 27.9 (SD=8.3) | 29.4 (SD=5.7) |
| **eCO Levels (ppm)** | | | | | | | | |
| Mean | 4.1* | 5.0* | 10.3 | 15.3 | 8.4 | 12.3 | 9.4 | 12.1 |
| Median | 4.0* | 5.0* | 8.0 | 9.0 | 7.0 | 9.0 | 7.5 | 8.0 |
| p-Value | | | 0.0002 | 0.0278 | 0.0009 | 0.0397 | 0.0061 | 0.0201 |

*One female (eCO=12) and male (eCO=6) were e-cig users for one year at the time of this study.

Table F.3. Smoking History & eCO Levels (ppm)

| **Smoking History** | **eCO (ppm)** | **Control** (N=21) | | **Subjects with Obstructive Lung Disease** (N=137) | | **Subjects with Restrictive Lung Disease** (N=50) | | **Subjects with Signs & Symptoms** (N=156) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Female | Male | Female | Male | Female | Male | Female | Male |
| Yes | Mean | 12.0 (n=1, eCig) | 6.0 (n=1, eCig) | 13.5 (n=10) | 27.5 (n=13) | 6.0 (n=1) | 23.8 (n=5) | 37.4 (n=5) | 23.8 (n=9) |
| | Median | 12.0 | 6.0 | 14.5 | 18.0 | 6.0 | 18.0 | 25.0 | 24.0 |
| No | Mean | 3.5 (n=15) | 4.8 (n=4) | 10.0 (n=63) | 12.1 (n=49) | 8.5 (n=28) | 8.8 (n=16) | 7.6 (n=75) | 10.5 (n=65) |
| | Median | 4.0 | 4.0 | 7.0 | 8.0 | 7.5 | 7.5 | 7.0 | 8.0 |
| Quit | Mean | 3.5 (n=2) | 2.5 (n=2) | 10.0 (n=28) | 14.0 (n=33) | 9.1 (n=10) | 7.4 (n=11) | 8.0 (n=20) | 9.5 (n=28) |
| | Median | 3.5 | 2.5 | 7.5 | 9.0 | 5.5 | 7.0 | 8.0 | 7.5 |

Table F.4. Use of Anti-Inflammatory Drugs & eCO Levels (ppm)

| **Use of Anti-Inflammatory Drugs** | **eCO (ppm)** | **Control** (N=21) | | **Subjects with Obstructive Lung Disease** (N=137) | | **Subjects with Restrictive Lung Disease** (N=50) | | **Subjects with Signs & Symptoms** (N=156) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Female | Male | Female | Male | Female | Male | Female | Male |
| Yes | Mean | N/A | N/A | 12.3 (n=33) | 13.3 (n=25) | 7.4 (n=7) | 25.0 (n=3) | 6.4 (n=13) | 18.3 (n=6) |
| | Median | N/A | N/A | 9.0 | 9.0 | 5.0 | 13.0 | 6.0 | 12.0 |
| No | Mean | 4.1 (n=16) | 5.0 (n=5) | 8.8 (n=42) | 16.7 (n=37) | 8.8 (n=22) | 10.2 (n=18) | 9.9 (n=69) | 11.5 (n=68) |
| | Median | 4.0 | 5.0 | 6.0 | 8.0 | 8.5 | 7.5 | 8.0 | 8.0 |

## APPENDIX G: SENSITIVITY AND SPECIFICITY OF ECO TEST RESULTS BY GENDER BY USING ECO=6

Table G.1. Test Results by **Obstructive Lung Disease** Status with Disease Prevalence of 62%, by Gender (= **Female**), not including subjects with restrictive lung disease (n=32). The sensitivity is 52% (58/111) and specificity is 62% (43/68).

| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present | Absent | |
| Positive | True positive (58) | False positive (26) | 84 |
| Negative | False negative (53) | True negative (43) | 95 |
| Total | 111 | 69 | 179 |

Table G.2. Test Results by **Obstructive Lung Disease** Status with Disease Prevalence of 74%, by Gender (= **Male**), not including subjects with restrictive lung disease (n=32). The sensitivity is 62% (69/112) and specificity is 50% (20/40).

| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present | Absent | |
| Positive | True positive (69) | False positive (20) | 89 |
| Negative | False negative (43) | True negative (20) | 63 |
| Total | 112 | 40 | 152 |

Table G.3. Test Results by **Restrictive Lung Disease** Status with Disease Prevalence of 24% by Gender (= **Female**), not including subjects with obstructive lung disease (n=223). The sensitivity is 45% (10/22) and specificity is 62% (43/69).

| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present | Absent | |
| Positive | True positive (10) | False positive (26) | 36 |
| Negative | False negative (12) | True negative (43) | 55 |
| Total | 22 | 69 | 91 |

Table G.4. Test Results by **Restrictive Lung Disease** Status with Disease Prevalence of 20% by Gender (= **Male**), not including subjects with obstructive lung disease (n=223). The sensitivity is 50% (5/10) and specificity is 50% (20/40).

| eCO Test Result | Disease Status (Gold Standard = PFT) | | Total |
|---|---|---|---|
| | Present | Absent | |
| Positive | True positive (5) | False positive (20) | 25 |
| Negative | False negative (5) | True negative (20) | 25 |
| Total | 10 | 40 | 50 |

# References


Altman DG, Bland JM. Diagnostic tests. 1: Sensitivity and specificity. BMJ: British Medical Journal. 1994 Jun 11;308(6943):1552.

Antuni JD, Ward S, Cramer DS et al. Exhaled carbon monoxide may be a marker of deterioration of lung function in cryptogenic fibrosing alveolitis and scleroderma. Am J Respir Crit Care Med 1999;159:A51

Antuni JD, Kharitonov SA, Hughes D et al. Increase in exhaled carbon monoxide during exacerbation of cystic fibrosis. Thorax 2000;55: 38-142.

Arif AA, Delclos GL, Lee ES, Tortolero SR, Whitehead LW. Prevalence and risk factors of asthma and wheezing among US adults: an analysis of the NHANES III data. European Respiratory Journal. 2003 May 1;21(5):827-33.

Baranamo DE, Rao M, Ferris CD, Snyder SH. Bilirubin reductase: A major physiologic cytoprotectant. Proc Natl Acad Sci USA 2002; 99: 16093-16098.

Babusikova E, Jesenak M, Durdik P, Dobrota D, Banovcin P. Exhaled carbon monoxide as a new marker of respiratory diseases in children. J Physiol Pharmacol. 2008 Dec 1;59(Suppl 6):9-17.

Bellamy, D., Booker, R., Connellan, S., & Halpin, D. Spirometry in practice. *A practical guide to using spirometry in primary care. The British Thoracic Society (BTS) COPD Consortium* 2005.

Biernacki W, Kharitonov SA, Barnes PJ. Exhaled carbon monoxide measurements can be used in general practice to predict the response to oral steroid treatment in patients with asthma. Am J Respir Crit Care Med 1999;59:A631.

Bousquet J. Global initiative for asthma (GINA) and its objectives. Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology. 2000 Jun;30:2-5.

Buist AS, McBurnie MA, Vollmer WM, Gillespie S, Burney P, Mannino DM, Menezes AM, Sullivan SD, Lee TA, Weiss KB, Jensen RL. International variation in the prevalence of COPD (the BOLD Study): a population-based prevalence study. The Lancet. 2007 Sep 1;370(9589):741-50.

CDC.gov. CDC - Asthma - Data and Surveillance 2018

Calhoun, W.J., Reed, H.E., Moest, D.R. and Stevens, C.A., 1992. Enhanced superoxide production by alveolar macrophages and air-space cells, airway inflammation, and alveolar macrophage density changes after segmental antigen bronchoprovocation in allergic subjects. *Am Rev Respir Dis*, *145*(2 Pt 1), pp.317-25.

Carmell E, Gleman R, Reznick A. The Biochemistry of aging muscle. Exp Gerontol 2002;7(4):477-489.

Chang C. Unmet Needs in Respiratory Diseases. Clin Rev Allergy Immunol. 2013;45(3):303–13. doi:10.1007/s12016-013-8399-2 PMID: 24293395

Chapman JT, Choi AM. Exhaled monoxides as a pulmonary function test: use of exhaled nitric oxide and carbon monoxide. Clinics in chest medicine. 2001 Dec 1;22(4):817-36.

Choi AM, Alam J. Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. American journal of respiratory cell and molecular biology. 1996 Jul;15(1):9-19.

Crapo RO. Pulmonary-function testing. New England Journal of Medicine. 1994 Jul 7;331(1):25-30.

Crystal RG, Gadek JE, Ferrans VJ, Fulmer JD, Line BR, Hunninghake GW. Interstitial lung disease: current concepts of pathogenesis, staging and therapy. The American journal of medicine. 1981 Mar 1;70(3):542-68.

Deveci SE, Deveci F, Açik Y, Ozan AT. The measurement of exhaled carbon monoxide in healthy smokers and non-smokers. Respiratory medicine. 2004 Jun 1;98(6):551-6.
Downs CA. Functional assessment of chronic obstructive pulmonary disease. JAm Acad Nurse Pract 2011;23:161–167.
Dworski R. Oxidant stress in asthma. Thorax. 2000 Oct 1;55(suppl 2):S51-3.
Evans SE, Scanlon PD. Current practice in pulmonary function testing. InMayo Clinic Proceedings 2003 Jun 1 (Vol. 78, No. 6, pp. 758-763). Elsevier.
Gajdócsy R, Horváth I. Exhaled carbon monoxide in airway diseases: from research findings to clinical relevance. Journal of Breath Research. 2010 Nov 30;4(4):047102.
GOLD (Global Initiative for Chronic Obstructive Lung Disease), Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease (updated 2014), 2014.
Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general US population. American journal of respiratory and critical care medicine. 1999 Jan 1;159(1):179-87.
Hayashi S, Omata Y, Sakamoto H et al. Characterisation of rat heme oxygenase-3 gene. Implication of processed pseudogenes derived from heme oxygenase-2 gene. Gene 2004;336:241-250.
Horvath I, Loukides S, Wodehouse T, et al. Increased levels of exhaled carbon monoxide in bronchiectasis: a new marker of oxidative stress. Thorax 1998a;53:867–870.
Horvath I, Donnelly LE, Kiss A et al. Raised levels of exhaled carbon monoxide are associated with an increased expression of heme oxygenase-1 in airway macrophages in asthma: a new marker of oxidative stress. Thorax 1998b;53:668-672.
Horvath I, MacNee W, Kelly FJ, Dekhuijzen PN, Phillips M, Döring G, Choi AM, Yamaya M, Bach FH, Willis D, Donnelly LE. "Haemoxygenase-1 induction and exhaled markers of oxidative stress in lung diseases", summary of the ERS Research Seminar in Budapest, Hungary, September 1999. European Respiratory Journal. 2001 Aug 1;18(2):420-30.
Inoue S, Suzuki M, Nagashima Y, Suzuki S, Hashiba T, Tsuburai T, Ikehara K, Matsuse T, Ishigatsubo Y. Transfer of heme oxygenase 1 cDNA by a replication-deficient adenovirus enhances interleukin 10 production from alveolar macrophages that attenuates lipopolysaccharide-induced acute lung injury in mice. Human gene therapy. 2001 May 20;12(8):967-79.
Johnson JD, Theurer WM. A stepwise approach to the interpretation of pulmonary function tests. Am Fam Physician. 2014 Mar 1;89(5):359-66.
Kacmarek, Robert, James Stoller, Albert Heuer. *Egan's Fundamentals of Respiratory Care, 10th Edition*. Mosby, 2017, pp. 418.
Kanazawa H, Kurihara N, Hirata K, Takeda T. The role of free radicals in airway obstruction in asthmatic patients. Chest. 1991 Nov 1;100(5):1319-22.
Keyse SM, Tyrrell AM. Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts of UVA radiation, hydrogen peroxide and sodium arsenite. Proc Natl Acad Sci USA 1989;86:99-103.
Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. American journal of respiratory and critical care medicine. 2001 Jun 1;163(7):1693-722.
Kharitonov SA, Barnes PJ. Biomarkers of some pulmonary diseases in exhaled air. Biomarkers 2002;7:1-32.
Kharitonov SA. Exhaled markers of inflammatory lung diseases: ready for routine monitoring? Swiss Med Wkly 2004;134:175-192.
Khatri K, Kaufman R, Baigelman W. Utilization of pulmonary function tests by primary care internists in a community hospital. American Journal of Medical Quality. 1994 Jun;9(2):49-53.

MacNee W. Oxidative stress and lung inflammation in airways disease. European journal of pharmacology. 2001 Oct 19;429(1-3):195-207.
Maines MD, Trakshel GM, Kutty RK. Characterization of two constitutive forms of rat liver microsomal heme oxygenase: Only one molecular species of the enzyme is inducible. J Biol Chem 1986;261:411-419.
Mandrekar JN. Simple statistical measures for diagnostic accuracy assessment. Journal of Thoracic Oncology. 2010 Jun 1;5(6):763-4.
Mafort TT, Rufino R, Costa CH, Lopes AJ. Obesity: systemic and pulmonary complications, biochemical abnormalities, and impairment of lung function. Multidisciplinary respiratory medicine. 2016 Dec;11(1):28.
Masoli M, Fabian D, Holt S, Beasley R, Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004 May;59(5):469-78.
Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Enright P, van der Grinten CM, Gustafsson P, Jensen R. General considerations for lung function testing. European Respiratory Journal. 2005 Jul 1;26(1):153-61.
Montuschi P, Kharitonov SA, Barnes PJ. Exhaled carbon monoxide and nitric oxide in COPD. Chest 2001;120:495-502.
Nath KA, Grande JP, Haggard JJ, Croatt AJ, Katusic ZS, Solovey A, Hebbel RP. Oxidative stress and induction of heme oxygenase-1 in the kidney in sickle cell disease. The American journal of pathology. 2001 Mar 1;158(3):893-903.
Ohrui T, Yasuda H, Yamaya M, Matsui T, Sasaki H. Transient relief of asthma symptoms during jaundice: A possible beneficial role of bilirubin. J Exp Med 2003;99:193-196.
Otterbein LE, Mantell LL, Choi AM. Carbon monoxide provides protection against hyperoxic lung injury. Am J Physiol Lung Cell Moll Physiol 1999;276:L688-694
Paredi P, Leckie MJ, Horvath I et al. Changes in exhaled carbon monoxide and nitric oxide levels following allergen challenge in patients with asthma. Eur Respir J 1999a;13:48-52.
Paredi P, Shah PL, Montuschi P, Sullivan P, Hodson ME, Kharitonov SA, Barnes PJ. Increased carbon monoxide in exhaled air of patients with cystic fibrosis. Thorax. 1999b Oct 1;54(10):917-20.
Paredi P, Kharitonov SA, Leak D, Ward S, Cramer D, Barnes PJ. Exhaled ethane, a marker of lipid peroxidation, is elevated in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2000 Aug 1;162(2):369-73.
Paredi P, Kharitonov SA, Barnes PJ. Analysis of expired air for oxidation products. Amer J Resp Crit Care Med 2002;166:31-37.
Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi RE, Coates A, Van Der Grinten CP, Gustafsson P, Hankinson J, Jensen R. Interpretative strategies for lung function tests. European respiratory journal. 2005 Nov 1;26(5):948-68.
Pepys MB, Hirschfield GM. C-reactive protein: a critical update. The Journal of clinical investigation. 2003 Jun 15;111(12):1805-12.
Petty TL: Scope of the COPD problem in North America: Early studies of prevalence and NHANES III data: Basis for early identification and intervention. Chest 2000;117:326S–331S.
Petty TL. Simple office spirometry. Clinics in chest medicine. 2001 Dec 1;22(4):845-59.
Pizzichini E, Pizzichini MM, Efthimiadis A, Dolovich J, Hargreave FE. Measuring airway inflammation in asthma: eosinophils and eosinophilic cationic protein in induced sputum compared with peripheral blood. Journal of allergy and clinical immunology. 1997 Apr 1;99(4):539-44.

Pretto JJ, Brazzale DJ, Guy PA, Goudge RJ, Hensley MJ. Reasons for referral for pulmonary function testing: an audit of 4 adult lung function laboratories. Respiratory care. 2013 Mar 1;58(3):507-10.

Rennard, S. I. (1999). Inflammation and repair processes in chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine*, *160*(supplement_1), S12-S16.

Rennard SI. COPD: overview of definitions, epidemiology, and factors influencing its development [review]. Chest 1998;113(4 Suppl):235S– 41S.

Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. New England Journal of Medicine. 2000 Mar 23;342(12):836-43.

Ruppel GL, Enright PL. Pulmonary function testing. Respiratory care. 2012 Jan 1;57(1):165-75.

Ryter SW, Choi AM. Carbon monoxide in exhaled breath testing and therapeutics. Journal of breath research. 2013 Feb 27;7(1):017111.

Sato S, Nishimura K, Koyama H, Tsukino M, Oga T, Hajiro T, Mishima M. Optimal cutoff level of breath carbon monoxide for assessing smoking status in patients with asthma and COPD. Chest. 2003 Nov 1;124(5):1749-54.

Saha S, Brightling CE. Eosinophilic airway inflammation in COPD. International journal of chronic obstructive pulmonary disease. 2006 Mar;1(1):39.

Sedgwick JB, Geiger KM, Busse WW. Superoxide generation by hypodense eosinophils from patients with asthma. Am Rev Respir Dis. 1990 Jul 1;142(1):120-5.

Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043-1046.

Tenhunen R, Marver HS, Schmid R. Microsomal heme oxygenase characterization of the enzyme. Journal of Biological Chemistry. 1969 Dec 10;244(23):6388-94.

Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, Stockley RA. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. American journal of respiratory and critical care medicine. 2013 Feb 15;187(4):347-65.

Willis D, Moore AR, Frederick R, Willoughby DA. Heme oxygenase: a novel target for the modulation of inflammatory response. Nature medicine. 1996 Jan;2(1):87.

Wood LG, Gibson PG, Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. Eur Respir J 2003;21:177-186.

Yamaya M, Sekizawa K, Ishizuka S et al. Exhaled carbon monoxide in exhaled air of subjects with upper respiratory tract infections. Am J Respir Crit Care Med 1998;158:311-314.

Yamaya M, Sekizawa K, Ishizuka S et al. Exhaled carbon monoxide levels during treatment of acute asthma. Eur Respir J 1999;13:757-760.

Yamaya M, Hosoda M, Ishizuka S, Monma M, Matsui T, Suzuki T, Sekizawa K, Sasaki H. - Relation between exhaled carbon monoxide levels and clinical severity of asthma. Clin Exp Allergy 2001;31:417-422.

Zanconato S, Scollo M, Zaramella C, Landi L, Zacchello F, Baraldi E. Exhaled carbon monoxide levels after a course of oral prednisone in children with asthma exacerbation. Journal of allergy and clinical immunology. 2002 Mar 1;109(3):440-5.

Zayasu K, Sekizawa K, Okinaga S et al. Increased carbon monoxide in exhaled air of asthmatic patients. Am J Respir Crit Care Med 1997;156:1140-1143.

Zhang J, Yao X, Yu R, Bai J, Sun Y, Huang M, Adcock IM, Barnes PJ. Exhaled carbon monoxide in asthmatics: a meta-analysis. Respiratory research. 2010 Dec;11(1):50.